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THE NATURE OF THE CANCEROUS PROCESS.*

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You have chosen a most important question for discussion this evening; a question which, for some years, has formed an active topic of discussion in the laboratories for research. Although the importance of the development of tumors in man has been known and commented upon for ages in the medical literature, it is of comparatively recent date that our methods of diagnosis have become sufficiently accurate, to undertake a systematic study of their nature. For this reason it is not possible to place a great deal of reliance upon the studies of malignant tumors in the literature prior to the writings of Virchow. Virchow, the ever observant pathologist, was keen to study the anatomical and histological features of new growths, and from him we have obtained a knowledge of many varieties of tumors which previously had gone unrecognized, or which were given no classification. And so for some years the work went on, tumors were described for almost every part of the body. Little by little, each author added some new points in his observations, until, at the present day, we have amassed a descriptive literature which covers fairly well the macroscopical and microscopical characters of new growths.

Naturally, with the close observations which the

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faithful observers made, many theories were propounded concerning the mode of origin and the development of new growths. Many of these have fallen by the way, and but a few are still to be considered. There are but few theories governing any of the questions of the sciences which, not based upon actual observations and fact, have withstood the onslaught of later investigation.

Even to-day I hesitate to give you the theories concerning tumors, but I wish rather to discuss the observations that have been made concerning the processes of tumor growth.

At the very outset we are confronted by a question of monumental importance to our subject, namely, what constitutes a tumor? Though in general terms the word expresses any unusual increase in size or swelling of a tissue, we have come to use the word in a more restricted sense. Swellings resulting from inflammation, collections of fluids or gas, and physiological development are not included in the meaning of the term; but we wish to refer to the local increase of one or more tissues in the sense in which Thoma spoke of "autonomous tumors." Such neoplasms have a law governing themselves, both as to their development and function.

However, a hard and fast line does not separate neoplasms from other instances of localized overgrowth of tissue. There is a gradual transition from inflammatory processes, hyperplasias, fibrous to autonomous tumors. Examples of the first instance, however, are segregated, on account of a known cause of their origin. Nevertheless, we cannot lightly overlook the close similarity in appearance—along with the difficulty of classification—of certain inflammatory conditions with those of new growths.

Borst describes tumors as excessive growth of tissues with autonomous characters, while all other growth of tissues, (inflammatory, regenerative, or hyperplastic) has a certain relation to the economy of the body. These forms of growth represent a type of reaction of tissues, in some instances being compensatory to wear and tear, in others to irritating stimuli. Neoplasms, on the other hand, occur, as far as we may judge at the present day, as spontaneous developments of tissue having no relation to the needs of repair or function of the other parts of the body. Thus, a tumor is a spontaneous atypical organ, not possessing the structure or function of any tissue or group of tissues otherwise found in the normal body—or as White puts it, "a tumor proper is a mass of cells, tissues, or organs resembling those normally present, but arranged atypically. It grows at the expense of the organism without at the same time subserving any useful function." The definitions of Rindfleisch, Lubarsch, Ribbert, and Birch-Hirschfeld agree in the main with this description. Ziegler adds to it, that tumors present no typical limit of growth. In all of these definitions the causative factor leading to tumor development is not considered—or, if considered at all, is regarded as spontaneous. Cohnheim, however, insisted that such atypical productions of tissue were erratic developments of embryonic deposits.

Difficult as it is to properly define tumor development, a still greater difficulty confronts us in classifying tumors. It was only after the histological nature of different tumors was well established, that comprehensive classification was attainable. For many, such a classification based on the histological appearance is still considered adequate, and is very simple. Tumors exhibiting an internal arrangement of their cells like that of a glandular structure are grouped together under the carcinomata, while tumors whose cellular and vascular arrangement resembles the connective tissue stroma of the body are classed as sarcomata. This simple classification which is based only upon the microscopical characters of thin sections takes no cognizance of the origin or development of the individual cells.

The embryologists have to-day established fairly well the origin of the individual cells of each organ. They have demonstrated that in the development of the single cell, the ovum, various stages or transitions are passed, and that from the earliest mass of undifferentiated cells, the morula, three layers of cells are produced whose function becomes fixed—as also the functions of the organs developed from each. In other words, from the three layer stage of the embryo onward, the cells have a definite rôle allotted to them. Now, as it became possible to group the tissues of the body according to their origin from one or other of the primitive layers, so also was it recognized (Waldeyer) that tumors could be classified in the same way. Hence, we may speak of tumors of the epiblastic, mesoblastic, and hypoblastic type. As the epiblastic and hypoblastic tissues resemble each other so closely, the tumors arising from them are considered together (Adami).

This classification, having an embryological basis, received some opposition. It was pointed out that, whereas on embryological grounds some tumors of the kidney histologically resembled tumors of glandular organs—adenomata and carcinomata—yet for embryological reasons, these would be placed among the connective tissue neoplasms. In this the nomenclature has helped the confusion, and we still find authors discussing the same tumor, the one as carcinoma, the other as sarcoma. Similar examples ot confusion may be given in other tissues; notably the glial and ovarian new growths. Adami has some years ago succeeded in harmonizing the two main features in tumor classification, in making the embryological derivation of the tissues the basis, while the histological characters constitute the subclasses of the tumor groups.

But what is still more important to the clinician than the scientific classification of neoplasms, is the question of malignancy. To the clinician, the great bulk of tumors are arranged into two great groups —the benign and the malignant. To him, these errors have a special meaning, indicating, on the one hand, a destructive infiltrating new growth which produces cachexia and metastases; on the other hand, the benign, a new growth with circumscribed boundaries, having no direct effect on the health, and producing no metastases.

The term "malignant," then, means something very definite to the physician and surgeon, and is, in fact, a clinical term. Not only are the production of cachexia and metastases to be feared, but removal of the primary focus does not necessarily ensure cure of the disease.

Is it possible for the pathologist to lay such hard and fast rules, differentiating malignant and benign neoplasms? Or, putting the question in a more direct way, is it possible by a histological examination to distinguish every malignant tumor from the benign? C. P. White in answering this question speaks guardedly, believing that "it is possible to allot any tumor a place in the classification, provided that we are sure of what we see under the microscope." Here is just the rub. At times we meet with tumors in which it is impossible to distinguish certain cells. Endothelial cells may look like epithelial cells; some inflammatory cells closely resemble the cells of connective tissue tumors; and lastly, we have learned by experience that certain tumor tissue which had the character of benign adenomata, had the vegetative characters of carcinomata. In short, the examination of a tissue section is *not* an infallible test for the malignancy of a tumor. One of the more constant histological features of malignant new growth, is the tendency of the tumor cells to infiltrate the neighboring tissue, and, if we have the opportunity of examining the borders of a neoplasm, the diagnosis is more easily made. This infiltrative character of cancers gives it the clinical features of fixation to the surrounding tissues.

Nevertheless, the broad principles of tumor growth and structure, which can be studied under the microscope, are usually a good guide in the diagnosis.

Difficult as is the recognition and diagnosis of some of the mature growths, it becomes still more difficult to recognize the earliest beginnings of tumor formations. If we are correct in surmising that the malignant tumors are the result of proliferation of one or a few atypical cells, how very minute is the earliest focus of such a neoplasm! It is quite beyond our present methods to recognize these aberrant cells until they have developed to form a definite mass. Nevertheless, such a mass of tissue has from the beginning the stamp of malignancy; the power to grow in limitless boundaries is inherent in each cell from the start. Seldom, does it seem, that tumor cells arrange themselves in an orderly manner, as in benign growths, and then later break loose in their vegetative characters.

To understand the growing power of an individual cell, it is well to appreciate the potential energy which they possess. Every normal cell, we may say, is endowed with energy which may be utilized by the cell for two purposes—growth and function. In the embryo, this cell energy is mainly devoted to cell reproduction, while the functions of the cell are more or less dormant. In the fully mature individual, these properties of the cell balance each other more closely—in all probability the cell utilizing more energy to carry on its individual function; while little energy, save in repair, is expended in cell proliferation. In tumor growths, on the other hand, we find the cells returning to their early embryonic characters, and instead of utilizing a given amount of the energy in carrying out the physiological functions, this energy is stored up and given over to the processes of growth. Hence, though the potential energy of a tumor cell may not be greater than that of any other cell, this power is spent in one direction—that of growth.

Exactly what conditions determine the direction in which a tumor cell utilizes its energy are still unknown to us. True it is, nevertheless, that each individual possesses a natural control over the rapidity of growth of all his body cells. This control, however, may be disturbed locally and even widely in the body by different means.

As all tumors increase in size by the proliferation of their own cells, the surrounding tissues take no part in the proliferative activity. Thus, as the tumor increases, it may push aside the cells of the part, or even destroy them, but these surrounding tissues do not take up the malignant nature from contact. With the advance of the tumor growth, lymphatics and blood channels are invaded, and as time goes on, there is the greater probability that more distant organs will be invaded. It is thus obvious that the earlier the recognition of malignancy is made, the more effective will be the operative measures to secure eradication.

In their exhaustive studies on the transplantation of tumors, Ehrlich and Apolant have made some interesting observations. It is found that many spontaneous tumors (cancers) of white mice can be transplanted from one animal to another, but all the inoculations of this tumor tissue into another animal, do not "take." For a given tumor it is found that the percentage of "takes" (successful transplants) is fairly constant, but differs from a tumor of another source. Thus, they have found that tumors possess a certain virulence, depending upon the ease with which they may be transplanted and upon the rapidity with which the tumor tissue develops in the new host. This virulence, too, is a variable quantity, and can be increased by repeated successful transplants. Ehrlich has further demonstrated that animals, which have been successfully inoculated with a tumor mass, will resist the second inoculation of new tumor tissue. In other words, these experimental animals have developed a certain immunity. It is still undecided in what form this immunity presents itself, though Ehrlich believes that the resistance is developed in consequence to the diminished amount of available nutriment for the second tumor. He contends that the growth of the first neoplasm has used up all the materials necessary for the active and rapid growth of tumor tissue. Others believe that a new substance is developed within the animal which protects it against further invasion. Possibly the future has more in store for us concerning immunity to tumors.

Concerning the parasitic theory of cancer, we need not open the discussion further than saying that there is no reason for believing that a specific virus exists.

Little as we know respecting the causation of cancer, we must approach this subject with an open mind, and not be over impressed with the traditional stereotyped hypotheses of Cohnheim and Ribbert. Possibly the embryonic rest theory of Cohnheim does account for a number of malignant and other tumors in early life; possibly, too, Ribbert's theory of dislocation of epithelial masses by inflammation of the neighboring connective tissue explains the origin of a few; but as Bashford points out, the study of the incidence of cancer among the various races of man demonstrates that the generalization of the embryonic theory is incorrect. Bashford has found that some relation exists between irritants of various kinds and cancer. In the civilized world epithelioma of the abdomen is unusual, while this form of cancer is frequent in Kashmir. In the hilly districts of Kashmir the natives wear earthenware charcoal ovens about their waists, and receive frequent burns about these parts. At these areas of repeated burns, epithelioma is prone to develop. We are familiar also with the cancers developing at the site of x ray burns. Again, in Ceylon and India, women suffer from epithelioma of the mouth in greater frequency than do women elsewhere. This is probably not due to a developmental difference in these women, but to the fact that Indian women chew betelnut and sleep with the plug in the cheek at the site where the cancer begins (Bashford). Other instances of race habits associated with the development of cancer can be cited.

The occurrence of cancer in association with chronic irritation has long been recognized, but at the present time more accurate observations are necessary to determine the part played by the various irritants. Even to-day we have sufficient information to indicate that these various irritants have little in common, save in acting as stimuli to cell growth. But the more we observe the association of irritation to cancer, the less prominent does the embryonic rest theory stand in the causation of cancer.

Still, to the practitioner the most important question hinges upon an early diagnosis. In general, it might be said that by the time a neoplasm has attained a size, recognizable to either patient or physician, it has assumed characteristics by which it may be recognized under the microscope as benign or malignant.

ON THE LARGE WHITE OR SOAPY KIDNEY.*

Oskar Klotz.

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Frerichs in 1851, from his observations on the kidneys, noted that "in general the fat content of the kidney in Bright's disease is less than what one would expect from the microscopic appearance, and one must not consider all the droplets in the epithelial cells as fat."

Many have drawn attention to the fact that what appear to be fat droplets in the epithelial cells of the renal cortex are not really such, and do not give the reaction for the neutral fats of the higher series.

In the careful analyses of Rosenfeld and also of Orgler, they independently came to the conclusion that the apparently fatty degeneration of the kidney as it is seen in the large white kidney, does not contain more, but actually less fat than normal. Stoerck comes to similar conclusions, and declares that the constituent of the cortical cells giving them their white color is protagon.

Various are the reports concerning crystalline bodies found in the kidney, but little information can be gained from those described from microscopical sections alone. Much of the confusion concerning these bodies has developed from the varied interpretations of the bodies and their microchemical reactions. Globular lipoid bodies have been jumbled together, into the class of myelins, other crystalline bodies are listed under protagons, while still a third variety of fine acicular crystals have remained undefined. I am here referring only to substances within the epithelial cells of the cortex or lying in the interstitial tissue.

In 1854 Virchow noted in the scrapings of diseased lungs and in the expressed mucus, peculiar round and transparent bodies which under the ordinary microscope exhibited a double contour. Bodies of the same nature he also observed

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in the spleen, thyroid, egg-yolk, testicle, ovary, pus and bile. His attention was still further attracted to this substance in finding that its reaction to simple reagents showed a similarity to that of the sheath of medullated nerves. To the substances in general he gave the name, "Markstoff" or Myelin.

Myelin, in Virchow's description, was a substance occurring often in globular form, but which on the addition of water swelled up and assumed various shapes with double contours. The substance he reported was readily soluble in hot alcohol, ether, chloroform, and turpentine.

Previous to Virchow, Henle had noted these peculiar bodies in the sputum and considered that they were fat, while Funke and Meissner had described similar structures and referred to them as corpora amylacea.

Liebreich undertook a chemical, or rather a microchemical analysis of the substance in question, and came to the conclusion that myelin was a mixture of the decomposition products of protagon with protagon itself. Diakanow, on the other hand, held that myelin was a chemical combination of protagon with lecithin, and he reported that the chemical reactions of such a combination were identical with those of myelin.

Following this several more accurate chemical analyses were made of brain and nervous tissue, isolating therein lecithin, neurin, cerebrin, protagon, cholesterin, and others. Many of these substances have been reported as being associated with the constitution of myelin, but these mixtures and compounds do not correspond to all the types of substance which Virchow designated as myelin. No definite or distinctive chemical reaction has been found to differentiate myelin from other substances, although many chemical properties have been determined — which when taken together constitute an equation signifying myelin.

Beneke examined materials showing myelin bodies, in various organs, and he noted that the substance was to be found in processes of degeneration where cholesterin was present. From this he concluded that, without cholesterin, myelin bodies do not occur. This was, however, demonstrated not to be the case. Neubauer and also Kohler found that myelin forms could be developed outside the body by allowing oleic acid and ammonia to run together. The substance, an ammonium oleate, had the microscopic properties of myelin as it is seen in the tissues, and they concluded that myelin was not a definite chemical body, but that it might be a substance of varying composition. This conclusion was based on the fact that besides the ammonium oleate a combination of oleic acid with other bases produced similar results.

Panizza denied the occurrence of myelin as a chemical entity and believed that the presence of a semifluid mass (dickfluessige Masse) in a liquid showed bodies similar to myelin forms. In short, he considered that myelin was only a physical state. He further stated that myelin occurring in the sputum was mucin, and was developed in the epithelium of the respiratory tract.

It is evident that Panizza based his studies upon the microscopical appearance of certain droplets within and outside of the cells. Certain known chemical reactions were not at his command and from the unstained microscopical appearances he has confused bodies of different chemical composition.

Small globules of refractile substances, having the property of swelling in water and showing a double contour at their periphery are as Panizza demonstrated of different kinds — but they are not all myelin. We do not wish to say that myelins are or must be of the same chemical composition, but they must be differentiated from mucins, fats, and certain other lipoid bodies.

Myelins are, under certain physical conditions, anisotropic, and when in this state they show peculiar figures by which they may be recognized. As, however, these same myelins may alter their optical characters and appear as isotropic granules or needle-shaped crystals, the polariscope alone cannot be depended upon to differentiate them from other substances. Nevertheless, the polariscope offers a ready method of determining in fresh and wet organs the presence of some of the myelins, and up to the present no other substances in the animal body have been determined which exhibit the same figures and optical characters as exhibited by the myelins. We must not, however, lose sight of the fact that other definite chemical bodies have been shown to possess the property of anisotropism and the stage of fluid crystals, and it may yet be shown that bodies of various kinds possessing these properties exist in the animal body. One such — protagon — I shall discuss later.

For the more extensive literature on the subject I refer the reader to the article by Adami and Aschoff. In their studies they observed that quite a variety of known chemical substances yield myelin forms of the anisotropic variety. Thus sodium, potassium and ammonium oleate; ammonium palmitate and stearate; cholesteryl oleate, palmitate, stearate and butyrate; a mixture of lecithin and cholesterin; cholin oleate; and mixtures of lecithin with ammonia, potassium hydrate or sodium hydrate, will all afford myelin forms of the anisotropic type. These authors were the first to point out that of these different chemical substances only those can be taken into consideration (as regards the myelins of the human system) which will retain their anisotropic myelin type at body, or at least room temperature. On this argument, the compounds of stearic and palmitic acids may be left out of consideration, and the only ones which may be considered are certain oleic acid compounds. Of these compounds they believe that cholesteryl oleate and cholin oleate play the most important part.

It is important to point out that Adami and Aschoff observed the presence of spheroliths as well as the fluid spherocrystals. The former were of two kinds, that of solid spheres and that of spherical masses of closely packed radiating acicular crystals, which might readily be distinguished from anisotropic fluid crystals in that they lacked the oily consistence of the latter. As I shall point out later, I was able to demonstrate that the anisotropic myelin forms (fluid crystals) may pass over into the anisotropic spheroliths with radiating needle-like crystals, in the process of dehydration. Adami and Aschoff were able to produce similar results by the action of formalin on cholin oleate.

Quite similar observations were made by Loehlein and later by White. They found that frozen sections of formalin-preserved tumors showed the presence of fine acicular crystals which when warmed became converted into globules showing anisotropism.

Kaiserling and Orgler and others have found that anisotropic globules are normal constituents of the adrenal. White notes that the crystals and globules in the tissues in some cases can be shown to contain cholesterol and a fatty acid, which becomes separated on warming. In other cases the globules show no evidence of fatty acid. The presence of the cholesterol in these globules was indicated only by the brown color in these globules when the tissues were treated by eighty-five per cent of sulphuric acid, while the fatty acid was demonstrated by staining a section with nile blue, and obtaining a blue color in the globules. A brown discoloration of the tissues and fluids is almost constant in treating sections with eighty-five per cent of sulphuric acid, and this I have found is an uncertain test for cholesterol in tissues. The blue coloration obtained in staining sections with nile blue is not to be depended upon as indicating in every instance the presence of fatty acids, as the shade of the color is influenced by external circumstances.

Although this author has demonstrated the physical properties and variations of many chemical substances, and has shown that artificial myelin forms owe their characters to water, "acids" or "alcohols of crystallization," the relationship of these substances to those in the tissues is only made by analogy. No analysis or isolation of the anisotropic globules or myelin forms as they occur in the tissues is made.

The following investigation concerns only the so-called myelin bodies found in the kidney. By myelin forms I refer to those substances which appear as spherical or varied shaped bodies, having under the microscope a double contour at the borders, which have the property of swelling up when in contact with water, are soluble in alcohol, and which are or can be made to appear anisotropic under the crossed Nichols prisms.

The chemical reactions of the "myelins" as I have found them in the kidney I shall give below. As the identity of the myelins from the various organs has not been determined, the reactions here given and the inferences drawn do not apply to myelins derived from other organs. I am of the opinion that there are myelins of various kinds, which has led to the confusion now existing concerning their general reactions.

It was my endeavor to obtain the myelin bodies in as pure a state as possible, and to control each step in the work the various extracts were examined with the polarizing microscope.

Material. — The kidneys were obtained from cases suffering from chronic nephritis. The organs were of the large white variety and were typical examples of chronic parenchymatous nephritis. The cortex was thick and of a glistening white color, giving the impression of extensive fatty degeneration. The dark medulla contrasted sharply with the pale cortex. There was no outward evidence of chronic interstitial disease, though this was apparent in the microscopic sections.

The amount of fat that could be demonstrated in frozen sections stained with Sudan III. was quite the reverse to what was expected from the macroscopic appearance. Instead of finding extensive fatty deposits in the kidney tubules, little or no fat was present in the epithelial cells of the cortex or medulla. Only a few cells of fatty infiltration were present about the larger blood vessels. The white and fatty appearance seemed difficult to explain. Scrapings from the cortex examined under the polarizing microscope showed a great number of doubly refractile bodies, and this number was readily increased by warming the slide until dry and then exposing the smeared surface to flowing steam. In all appearances the kidneys were similar to those so extensively described by Stoerck.

In these kidney scrapings the anisotropic bodies were spherical and varied in size from two to ten microns. The appearance was the same in all, there being a dark cross placed on the illuminated sphere. If allowed to dry, the bodies kept their form, but the refractile portions of the bodies gradually showed the development of crystals, radiating from the center of the sphere. These radiating crystals were individually composed of needle-shaped crystals which, as the specimen became dehydrated, became massed together forming single, double, or quadruple sheaves. If these desiccated crystals were exposed to flowing steam for a few minutes or longer, they again assumed their original form of anisotropic globules.

The fresh kidney scrapings were treated with various reagents, and the results watched under the microscope. (1) When treated with absolute, ninety-five per cent, and eighty-five per cent alcohol the doubly refractile bodies disappeared, or at least lost their doubly refractile nature. On the evaporation of the alcohol amorphous isotropic granules appeared, which, however, on the addition of moisture and heat took on the characters of the anisotropic globules, save that now these globules were much increased in size, up to 20 μ. (2) When the scrapings were treated with neutral ether, the greater number of the doubly refractile globules lost their anisotropism, and on the evaporation of the ether, needle-shaped crystals were left behind in their stead. These acicular crystals were distributed in small masses or singly through the field. The needles were fairly long and tapered to fairly sharp points. The ether had another effect on the anisotropic globules when these were collected in masses. The addition of ether caused these globules to become stretched out into myelin forms with anisotropic ends showing the dark cross. These long myelin forms were quite similar to the appearance of those seen in brain tissue, or the "myelin-forms" produced by bringing semifluid sodium oleate in contact with ether. (3) When treated with excess water the anisotropic globules disappeared, but reappeared again on slow evaporation, or if heated to desiccation would reappear by exposure to steam.

Too much weight must not be placed on the microscopic absence or disappearance of these bodies, when treated with certain reagents, as denoting true solution. It is quite possible and even probable that the globules lose their anisotropic nature when in contact with certain substances, while, however, they do not become dissolved in the substance. It is most likely that the disappearance of the anisotropism of the globules when in contact with ether is of this nature. Frozen sections of some of the kidney tissue preserved in formalin did not show any of the anisotropic globules seen in the fresh scrapings, but in their stead acicular crystals were scattered through the organ, some appearing within vacuoles in the epithelial cells, others in the interstitial tissue.

At autopsy the greater portion of the kidney substance was freed from fat and the loose tissue of the capsule and pelvis. The kidney substance was then placed in a clean jar of neutral ether and allowed to stand for five days. At the end of this time the ether showed some absorption of the neutral fats. The aqueous juices of the kidney became expressed and formed a small quantity of brown fluid at the bottom of the jar.

On evaporating some of the filtered ethereal extract and exposing the dried slide to steam, the anisotropic globules were present in small numbers. Some fats and other extractives were also present in the evaporated residue.

Some of the watery layer at the bottom of the ether extract was collected, and slowly dried. This dried material showed innumerable anisotropic globules mixed with a pigment and débris. The addition of a little water to convert the residue into a pasty mass brought to light a great many more of the anisotropic globules. Occasional cylindrical myelin forms were present among the globules, and it was noted that the anisotropic globules could be changed to the cylindrical myelin forms by pressure on or dragging of the coverslip.

About one-fourth of one kidney was taken from the ether and ground up in a mortar. The ground material was then extracted with absolute alcohol (in excess), in the cold. The flask containing the mixture was frequently shaken and kept for five days at room temperature. The alcohol was then filtered clear. A small amount of the alcohol was evaporated, and the greasy looking residue was moistened with water and warmed. Many anisotropic globules appeared in the specimen. The globules were larger than those found in the original kidney scrapings.

A small quantity (one cubic centimeter) of the alcoholic filtrate was treated with chloroform in excess (ten cubic centimeters). A heavy white milkiness resulted which, when the fluid was allowed to stand, formed a scum at the top of the fluid. This scum under the microscope was made up of needle-shaped crystals, which had no definite arrangement to each other. It was found that if the chloroform alcohol mixture were evaporated as soon as the precipitate appeared no needle crystals were to be found in the fluid, but instead isotropic granules similar to those seen in the original kidney scrapings when treated with alcohol. Both the acicular crystals and the isotropic granules could be changed into anisotropic granules by moistening them with water and warming, or exposing them to warm steam.

The entire alcoholic filtrate was then precipitated with excess chloroform, and the resulting white precipitate was filtered out and washed with fresh chloroform. This residue on the filter was then dissolved in new absolute alcohol.

From the last alcoholic solution the various types (anisotropic globules, needle-shaped crystals, and isotropic granules) of residue could be obtained and each could be changed into the other form by heat, water, or chloroform.

When some of the reprecipitated (chloroform and alcohol) residue is treated with ether no solution of these crystals takes place. In the presence of ether the crystals arrange themselves radially or in double or quadruple sheaves. The radial masses are made up of fine needle-shaped crystals which under the polarizing microscope are isotropic. The arrangement of the crystals is quite like that of phenylglucosazone. When the ether is evaporated from the undissolved crystals, the latter can be converted into the anisotropic globules having the maltese-cross dark areas.

We are able, therefore, to obtain a recrystallized product from the alcoholic extract which contains anisotropic globules similar to those seen in the original kidney scrapings. From the reaction of ether on the isolated myelin bodies in their crystallized form, we must uphold the insolubility of the anhydrous form in ether. This is further proved in the following experiment. When some of the alcoholic extract was mixed with an excess quantity of neutral ether an opalescence immediately appeared, and a white flocculent precipitate resulted. A few doubly refractile globules were present in this precipitate which consisted mainly of amorphous isotropic granules. In the filtrate no anisotropic bodies could be demonstrated. That the globular anisotropic form of the substance disappears, to a great extent, in apparent solution when in contact with ether on a slide is to be demonstrated in each step of the experiments, but this conversion of the globular type of myelins into the long cylindrical type, the isotropic amorphous form or the crystalline product appears to be rather a physical change and not solution, and I would refer the reader to a paper on an analogous subject by Bayliss and Starling.

Anisotropic globules obtained on a slide by the evaporation of the alcoholic extract, gradually disappear when brought into contact with a two per cent solution of osmic acid. The gradual dissolution of these globules can be observed under the polariscope — and concurrently may be noted the blackening of the fluid. Differing from solution of the common oleates the blackening does not take place immediately, but requires some three to six hours for its completion. In making these reactions with osmic acid, and using fair quantities of the residue in a watchglass, the resulting color was always black and not the gray black spoken of by Kaiserling and Orgler. Nor was I able, after the myelin substances had become thoroughly blackened, to remove the color by the use of xylol or chloroform.

If the reaction with osmic acid be carried out in a solution, the fluid becomes blackened, and a black granular precipitate sinks to the bottom. This precipitate did not take any particular crystalline form.

For comparison, a pure sodium oleate was prepared. The pasty mass showed innumerable anisotropic globules and myelin forms. The addition of osmic acid to this preparation led to a gradual disappearance of the anisotropic globules, so that nothing could be seen of them. After a few minutes the solution became brown and then in a short time turned black. The dissolution and disappearance of the anisotropic globules was quite similar to that observed in the kidney extract. A black and structureless precipitate also developed in the test fluid, and anisotropic globules could not be obtained from the osmic acid mixture.

The similarity of the anisotropic globules in their reactions with perosmic acid to the globules present in sodium oleate paste was striking — the difference being in the length of time in which the blackening took place.

Tests for cholesterin carried out on the alcoholic solution, or on the crystalline precipitates therefrom, proved negative. Neither the Liebermann-Buchard nor the Neuberg-Rauchwegers reaction demonstrated the presence of this substance in the extracts, nor were cholesterin crystals ever encountered in the microscopic examination.

By accident a watchglass with some dried chloroform-alcoholic precipitate became immersed in boiling water. On removal from the water the granular precipitate had become converted into a gelatinous pasty mass. Anisotropic globules were not present in this mass.

Some of the alcoholic extract was treated with alcoholic solution of calcium chloride. This led to a marked opalescence with a succeeding flaky white precipitate. Warming the mixture hastened the formation of the precipitate. The addition of lead acetate to the watery extract led to a heavy white precipitate without the appearances of opalescence.

The anisotropic bodies when obtained on a slide from any of the previous cases and treated with two per cent acetic acid did not alter their shape or appearance.

When a concentrated sulphuric acid or a mixture of concentrated sulphuric acid and formalin (Goloditz' reaction) was brought in contact with the anisotropic globules under the microscope these globules disappeared as if in solution and no trace of them remained. There was no brown or black staining of the globules or the solution to indicate cholesterin. During the process, while the sulphuric acid was acting on the anisotropic globules, very fine particles were given off. These particles may have been fine gas bubbles, but this was not definitely determined.

When the anisotropic myelin globules are treated with fifteen per cent hydrochloric acid the globules quickly lose their anisotropism and disappear.

When the myelins (in the anisotropic form) have been isolated in a pure state and allowed to dry they are not dissolved in chloroform or ether. This apparent want of conformity with Virchow's postulates we refer elsewhere.

It has been pointed out by many that the admixture of oleic acid with cholesterin yields anisotropic globules which may take the type of the cylindrical myelin bodies. I have prepared such a mixture and can verify the observations of Adami and Aschoff and others. Such a mixture is soluble in alcohol, but the reactions of this solution differ from those of our alcoholic extract of the kidney. When treated with chloroform or ether, the alcoholic solution of the cholesterin oleic acid mixture remains unchanged and no precipitate is obtained by an excess of the reagents. In these solutions the cholesterin can readily be detected by dry or wet tests.

FATTY ACIDS. — A chemical examination was undertaken to determine the nature of the substance we were dealing with, and proof conclusive was obtained that fatty acids were present. Some of the alcoholic extract was precipitated with chloroform, and the precipitate was redissolved in absolute alcohol. To this alcoholic solution was added a one per cent hydrochloric acid solution until the whole was decidedly acid. This mixture was thoroughly shaken and after twenty-four hours was shaken out with neutral ether. The ether was then separated and given several washings with distilled water until no more hydrochloric acid could be detected in the wash-water. The ethereal solution was then tested as follows:

(*a.*) Several drops of the ether, when placed on glazed paper, left a permanent fatty stain.

(*b.*) When a small quantity of the ether was allowed to evaporate on a watchglass, clusters of isotropic acicular crystals appeared, which did not take any special arrangement to each other. Some oily globules were also present at room temperature. The acicular crystals melted when warmed at 50° C. and recrystallized at 49° C.

(*c.*) A faintly alkaline alcoholic solution of phenolphthalein became decolorized when treated with a small quantity of the ether residue.

(*d.*) Heating a small amount of the ether residue (by evaporation) in contact with acid potassium sulphate did not give the odor of acrolein.

(*e.*) When some of the ether residue was treated in the cold with a five per cent solution of potassium hydroxide and,

(1) A solution of calcium chloride was added to it, a flocculent white precipitate resulted.

(2) A solution of lead acetate was added to (*e*) a heavy precipitate resulted. This precipitate was partly soluble in ether.

(3) When dried, and the residue was dissolved in alcohol, a flocculent white precipitate resulted when the solution was acidified with a one per cent solution of hydrochloric acid, which precipitate was soluble in ether.

(4) When the potassium hydroxide mixture was slowly evaporated and warmed slightly, there appeared myriads of small anisotropic globules, similar to those noted in the early experiments and kidney scrapings, and showing the characteristic dark cross.

A quantity of the material was precipitated with lead acetate similar to that obtained in reaction (2). This precipitate was filtered, washed with distilled water and allowed to dry. This precipitate was now treated with ether and thoroughly extracted. The greater portion of the precipitate was found to have dissolved in the ether. The ether was again filtered and the filtrate allowed to evaporate. The residue from the ether evaporation was collected and suspended in distilled water and acidified with weak hydrochloric acid solution. The mixture was then shaken out with ether, and the ethereal extract separated. When this ethereal extract was evaporated small transparent globules remained on the watchglass. These globules were of an oily consistence at room temperature, but when placed on a salt and ice mixture became crystalline. The globules were soluble in alcohol, ether, and chloroform. When treated with a five per cent potassium hydroxide solution anisotropic globules were obtained, and the whole was soluble in water. The lead precipitate, which was not soluble in ether, was treated with a weak hydrochloric acid solution and the mixture shaken out with ether. On the evaporation of the ether a very slight amount of residue was obtained, which was made up of rather coarse isotropic crystals, arranged in small masses which, when heated, would melt at 61° C. These crystals were soluble in alcohol, ether, and chloroform, but were insoluble in water. When treated with a five per cent solution of potassium hydroxide and calcium chloride solution added, a white precipitate results. The material, however, was in too small a quantity to determine whether it was composed of one or more substances. Clear it was, though, that this substance belonged to the higher fatty acids. (The melting point of these crystals was, I believe, influenced by incomplete dissociation from oleic acid.)

That these fatty acids were in combination in our original alcoholic extract with a base or substances acting as bases, is clear from the behavior towards chloroform and ether, and the subsequent reactions.

The fatty acids which we have isolated, and which have been shown to form soaps in the presence of alkalis, occurred in a mixture, there being a portion which at room temperature appeared in a crystalline state, and another larger portion which was present in small oily globules. The latter, which had the properties of being in an oily state at room temperature, crystalline at 0° C., forming soluble salts with sodium and potassium, and whose lead salt was soluble in ether, was clearly demonstrated to be oleic acid.

With the small amount of material at our disposal, I was not able to determine with certainty the nature of the other fatty acids which were liberated.

BASES. — A determination of the bases separated from the fatty acids was also made. Calcium and magnesium were not present. On the other hand, sodium and potassium were found in fair quantities. (The various reagents used during the experiments were also examined for these substances with a negative result.) Proteid matter either free or in combination was not demonstrable.

A. Schmidt in 1897 determined chemically that myelins of the sputum were "protagon," and that these were normal secretions of the bronchial and tracheal mucosa. Besides protagon he also found small amounts of cholesterin and lecithin in these secretions.

In 1906 Stoerck published a most interesting research concerning the "protagons" of the large white kidney. In this organ he found quantities of "protagon" in the epithelial cells, as well as in the interstitial tissue, while occasionally the substance found its way into the lumina of the tubules.

His examinations were mostly of a histological character and the determination of the protagon was confined to frozen sections of fresh and formalized tissue. The appearance of the protagon in the latter sections was quite similar to the needle-shaped crystals which I found in the formalin specimens examined, while in the fresh tissue he encountered the anisotropic globules as we have described them above.

Stoerck found that these doubly refractile globules take the Sudan stain but faintly, and are soluble in alcohol, acetone, ether, benzine, chloroform, and xylol. I must again point out here that Stoerck determined the solubility of the substance under the microscope, and that rather than demonstrating that the anisotropic globules were soluble in the above-named substances he proved that they altered their optical characters — in some cases on account of solution. He found that these optically active substances were to be found normally in the adrenal, and are at times to be seen in tumors and in atheroma.

Panzer, who carried out the chemical analysis for Stoerck, found that the protagon which Stoerck was dealing with differed from the protagon of the brain, in that it was free from nitrogen and phosphorus. For true protagon (so-called), nitrogen and phosphorus are essential constituents.

It would seem to me that the substance Stoerck was dealing with is identical with the myelins referred to in this paper. There is at least great similarity in his histological findings and mine. On the other hand, we disagree in the matter of solvents, which I believe is due to the fact that Stoerck relied on the change in the (microscopic) optical activity as denoting solution.

Concerning the occurrence of "protagon" in the kidney Stoerck believes that it denotes a process of degeneration.

Panzer later made a more detailed study of the so-called protagon of the kidney. He isolated a substance by precipitation from cold acetone, and demonstrated that the crystals so obtained had anisotropic characters similar to the original globules in the kidney. He does not state, however, by what method the crystals were altered to the anisotropic globules. The crystals were found to be a compound of oleic and another fatty acid with cholesterin.

In Panzer's determinations, however, the possibility of the cholesterin remaining in the solution as a mixture cannot be ruled out, and it may be that this substance was not united with the fatty acids.

In a recent paper White has reported the occurrence of needle-shaped crystals in various tissues. For several of these he determined the melting point and by analogy of their characters has concluded their chemical composition.

It need hardly be mentioned that in the fresh kidney tissue crystals of various kinds are to be met with (tyrosin, urates, phosphates, "protagon" and others) while in the formalized tissue the varieties of crystals are further increased. The action of the constituents of the commercial formalin, containing formic and some acetic acid on the tissues, must not be forgotten. Hence, the deductions based on the crystalline bodies present in tissues preserved in this medium must be made with care.

The melting point of mixtures of fat or fatty acid crystals gives us no clue as to the constituents. It is only after the material is gained in its pure state that this test serves us a purpose. I can therefore not claim to have determined the exact nature of the second fatty acid isolated.

CONCLUSIONS.

To sum up, we have found that the large white kidney contains a compound, myelin, so-called, soluble in alcohol, from which it can be precipitated by chloroform. This precipitate with chloroform possesses the optical characters of the substance seen in the fresh organ. On analysis this substance contains fatty acids (oleic and other fatty acids being determined). These fatty acids were in combination with sodium and potassium, while neither proteids nor cholesterin could be demonstrated in the compound. The fatty acids when isolated, and again reunited with sodium and potassium, gave anisotropic globules similar to the original material.

It is to be pointed out that the large white kidney which we have usually considered as the fatty kidney, is in reality a soapy kidney and that part at least of the myelins contained therein consist of soaps of oleic acid. These fatty compounds are not readily demonstrated by the ordinary staining with Sudan III., but can be obtained by extraction with alcohol.

[I am greatly indebted to Professor Adami for the many suggestions he has given me in this work, and to Professor Aschoff for the kind assistance given me during my stay at his laboratories in Freiburg, Germany. I wish to thank Dr. Bruere for the kind assistance he has given me in making the chemical analyses.]

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EXTENSIVE SARCOMA OF THE HEART, INVOLVING THE BUNDLE OF HIS.

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Whatever be the real causation of the heart beat, and through whatever agency the rhythm of the heart is maintained, there is no doubt that recent studies of the anatomy of the heart itself have thrown considerable light on the subject, and the work of Tawara, His, Keith, and many others, has led one to believe that the explanation is nearing a solution.

Passing from the earlier authorities, whose views as to the heart's action were based on a belief that the blood itself, circulating through the chambers, was responsible for the individual beat, we were brought to consider the heart tissue as having some special function whereby the action, more or less automatic, was maintained in the absence of external control. When, however, the researches of anatomists and neurologists led us to see that ganglion cells and nerve fibers were present in abundance, the so-called neurogenic theory took its place as of paramount importance, and the heart's action was regarded as primarily subservient to the nervous mechanism of the body—the ganglia in the heart tissues originating the individual beats. Gaskell's researches, however, led to the myogenic theory, according to which the muscle cells themselves were possessed of a power independent of the nervous system, and by means of which stimuli originated and conduction and contractility were maintained.

When, however, ten years later (1893), Kent and His demonstrated special muscle bundles leading from the right auricle to the two ventricles, and with the further work of Keith, Flack, Erlanger, Gibson, Mackenzie, and many others, it was shown that the various functions of irritability, contractility, and conductivity depended in no small degree on the integrity of these muscular strands, a new light was thrown on the whole subject of cardiac rhythm. In cases of myocardial disease with arrhythmia it was frequently demonstrated that the course of these fibers was disturbed by anatomical lesions of the bundles, and that when this existed the causation was attributable to this localized pathological change.

It remains, however, yet for physiologists and anatomists to determine whether or not the circulatory changes in functions thus induced are, after all, merely to be associated with muscular change, for in more recent times Pankul, working under Kronecker, found in the very bundles of His, running throughout their substances, nervous plexuses, injuries to which might readily account for any functional disturbance in which conductivity of the fibers exists.

Quite recently Wilson has again demonstrated that the atrioventricular bundle contains not only a special form of muscle fiber, distinct from the ordinary myocardium, but that it is an important and intricate nerve pathway, in which are found: (1) ganglion cells, (2) nerve fibers, and (3) nerve plexuses in close relation to the muscle fibers of the bundle.

It is obvious, then, that the exact relation of the bundles to cardiac rhythm requires more elucidation, and that while possibly, and even probably, the explanation lies in the altered condition of some tissues in these muscles strands, yet the whole explanation is not complete.

That such is the case is even more emphasized by an opposite state of affairs, that is, when, with more or less complete destruction of the bundles of His, no change in cardiac rhythm is observed. Such a case has come to our notice, and the interference of conductivity seems so probable that it may be cited as a very fair type of case which might illustrate the possibility of some other factor yet unknown which is either responsible for the control of the heart's action, or which, in the absence of the usual means of exercising this function, may take up the work and maintain the regularity of the heart's action.

Extracts from Dr. Tull's Case Report:

A bank clerk, aged twenty-three years, was admitted on October 19, 1907, to the Royal Victoria Hospital, complaining of swelling of the face, feet, hands, and scrotum, and of shortness of breath. He was well until the end of April, 1907, when he suddenly fainted while sitting at his desk. He was unconscious only a few seconds, and remained for five or six days. He had had a cough and spat up a fair quantity of mucus, but no blood. His doctor thought his right lung was involved (tuberculous), and sent him to a hospital, where he remained ten days, and then after one week at home, he went to the mountains. There he remained from the middle of May to July, 1907. While there he began to have urgent dyspnœa on slight exertion. If he walked upstairs he was very dyspnœic, as also at night. With the dyspnœa there was cardiac palpitation, and his pulse varied from 100 to 140. No œdema or urinary disturbance was present at this time, and his cough was less troublesome. He returned to Montreal in July and was seen by one of us (Dr. Martin), who examined him under the x-rays, and found a large intrathoracic tumor mass. He went home for the summer to Prince Edward Island to rest; he kept fairly well, except for attacks of dyspnœa, until August, when his throat began to swell, and he had some dysphagia. His doctor did some minor operation on his throat, which relieved this symptom. Toward the end of August his left arm suddenly became swollen, and has remained so, more or less, up to the time of admission. About this time, too, his face began to be swollen in the mornings; no headache was present. Three weeks later his feet began to swell, and were very œdematous at night. At this time he had pain down his sternum, and especially just to the right of it. His doctor thought there was fluid in his pericardium and blistered his precordium with some relief.

He has had a rapid gain in weight since the onset of the anasarca.

On admission the patient was a well nourished young man of twenty-three years. There was puffiness of the eyelids, with slight pitting over the forehead. Superficial veins marked, especially over the upper thorax, and upper right arm, to a less extent in the lower thorax. Relative dulness in second space above, laterally from 4 cm. to the right of the right sternal border, 25 cm. to the left. The dulness at the base was extensive, measuring some 12 cm. transversely. Absolute dulness in the third space above. At the apex both sounds were loud and clear. The liver dulness extended 8 cm. below the costal border in the right nipple line, and the large firm organ could be felt extending across the epigastrium. Penis and scrotum were tremendously œdematous.

Diary. October 21, 1907. Condition unchanged. Dyspnœa. No pain. Fluoroscopic examination showed an extensive mediastinal shadow. The heart was very large, and extended far into the left axilla. At the base the dulness measured 27 cm. transversely. The heart beat was wave-like, as if adhesions were present. The mediastinal shadow at the base was quite wide. No pulsation. The diaphragmatic movements could not be made out.

November 6, 1907. Exploratory puncture made in right chest, posteriorly, and a clear fluid withdrawn. Microscopic examination showed no unusual cells. Increasing anasarca.

November 20, 1907. The larynx was examined by Dr. Birkett, who said that there was thickening of the false vocal cords. General condition was much weaker, but his heart sounds remained strong and regular. General anasarca was increasing.

November 24, 1907. Right chest aspirated; 25 ounces of hemorrhagic fluid withdrawn. Dyspnœa partially relieved.

November 25, 1907. Fluoroscopic examination showed mediastinal dulness to have extended considerably. There was a dense shadow all over the right chest, and on the left side this extended high up, as far as the second rib, and extended downward and outward into the left axilla, where the heart beat was seen, wave-like in character. Diaphragmatic movements were not visible. The patient died rather suddenly when moving about in a wheel-chair.

Clinical Diagnosis. Mediastinal neoplasm, sarcomatous; blocking of superior vena cava, with bilateral hydrothroax, especially on the right side; pulmonary œdema.

Pulse and Respiration.

		Pulse		Respiration.	
		A.M.	P.M.	A.M.	P.M.
October	19	76	84	—	24
"	20	76	80	20	20
"	21	72	72	24	22
"	22	72	76	24	24
"	23	76	76	30	28
"	24	72	72	28	32
"	25	80	90	28	28
"	26	78	72	28	24
"	27	76	72	30	24
"	28	80	76	30	28
"	29	80	72	30	28
"	30	72	76	28	26
"	31	84	88	30	28
November	1	82	88	30	30
"	2	84	88	28	30
"	3	88	76	30	20
"	4	80	80	30	26
"	5	88	72	30	24
"	6	78	76	32	28
November	7	76	80	28	36
"	8	82	96	30	30
"	9	76	84	30	30
"	10	76	80	32	26
"	11	78	80	30	28
"	12	80	80	28	42
"	13	92	72	28	26
"	14	78	76	30	31
"	15	76	100	28	44
"	16	76	78	28	36
"	17	76	72	30	28
"	18	84	72	28	28
"	19	84	84	30	28
"	20	72	96	28	36
"	21	80	72	30	28
"	22	80	74	30	36
"	23	80	72	30	36
"	24	72	90	28	31
"	25	62	88	30	36

The patient's condition was closely observed during his stay in the hospital. The extensive venous stagnation which was present in the upper portion of the body indicated a blocking of the superior vena cava, and venous tracings were out of the question. The pulse rate, as is indicated by the table, did not attract attention to organic involvement of the heart, and was in no way indicative of a Stokes-Adams disease. The character of the heart beat as seen with the fluoroscope was wave-like, appearing as if the heart were impeded by adhesions. Arrhythmia of the auricles and ventricles could not be observed at any time.

AUTOPSY. Body that of a young man of large frame, with great œdema and hard brawniness of both arms, chest, abdomen, back, and both legs; scrotum enormously enlarged and hard; penis greatly œdematous; some œdema of face and neck; pupils equal, not contracted; body warm; beginning lividity of right side of chest, and of back; early bedsores on right hip; prominence of right hypochondrium; on section, tissues were very œdematous and bloody; much ascites with turbidity.

Neck. The jugular veins were thrombosed on both sides at the height of the larynx, and extending down from here the carotids and jugulars, just above the clavicle, became enveloped by tumor.

Below the clavicle the tumor formed one large mass behind the sternomastoids and enveloped, laterally, the organs of the neck; the tumor mass was three and one-half inches wide, solid, and of a light pink color; from here it passed into the mediastinum, lying in the middle line until it reached the pericardium, whose upper (visceral) surface was covered as far as the upper three-quarters of the heart. Parietal pericardium over apex was clear; the rest of it involved, circularly. The tumor mass also extended to the right over the parietal pleura, on to the diaphragm, the whole of which, on the right side, was infiltrated, the left being clear. The peritoneal surface of the diaphragm showed the tumor adherent to the liver. The arch of the aorta was surrounded by tumor, the descending thoracic aorta was not involved. This part of the tumor was a continuation of that affecting the parietal pericardium. The larynx was œdematous along the aryepiglottic fold; the trachea was not apparently compressed; although the tumor mass surrounded it at the bifurcation, the angle of the bifurcation was a mass of tumor tissue. Trachea and bronchi were clear. The thyroid was pale, showed colloid, not enlarged.

Thorax. On section of the thorax, the sternum was found adherent to the underlying white mass of fleshy consistence—new growth of mediastinum. The sternum was infiltrated and broke easily. On removal, the mass occupied the right two-thirds of the chest cavity. There were adhesions of the left lung posteriorly, on the upper lobe. The chest contained several hundred cubic centimeters of bloody fluid. There was excess of fluid in the pericardium, about 200 c.c.. The upper part of the heart, anteriorly, was covered by the pericardial growth. The right lung was completely adherent and could not be separated from the diaphragm.

Left Lung. The apex of the lung had tumor tissue growing over it, on the pleural surface, while some tumor tissue infiltrated the substance of the lung beneath. There were some adhesions of new growth over the pericardium where the infiltration of the lung was seen. The lung was gray, crepitated throughout, save for a small patch of hazel-nut size, at the anterior margin of the base; this, on section, appeared red, like an infarct, and was solid. No sign of tuberculosis in the lung. On section, the lung was dry and healthy looking.

Right Lung. Bullæ of œdema between pleural surfaces—cysts of pleura. The lung was solid, meaty, and much clear fluid could be expressed from it. There was no true infiltration of newgrowth in the organ. On section there was œdema.

Heart. This presented a remarkable appearance; the organ was covered by a cap of tumor over the entire right auricle and one-half of the right ventricle. This subpericardial mass also extended over the left auricle and slightly onto the left ventricle. In the left ventricle a solid mass of tumor could be felt within the muscle tissue.

On opening the left ventricle the interventricular septum was found to be a mass of newgrowth, save at its very apex; the septum was much thickened, averaging half an inch. On one side of the right ventricle the columnæ were partly obliterated by the huge plate-like growth of the tumor. The endocardium was everywhere smooth. All the valves appeared to be healthy, and no definite stenoses could be made out. The muscle tissue, apart from the tumor, was very pale. One of the coronary vessels was cut across and was seen to be embedded in tumor, and showed thrombus on its walls. A second cap of sarcomatous tissue was formed by the parietal pericardium, which, in its upper part, was fused into one solid mass of tumor tissue, continuous with the anterior mediastinal tumor. This extended in the form of a cap over the heart. The superior vena cava was firmly surrounded with newgrowth, while its lumen was blocked by a thrombus of some long standing. The main vessels of the neck, arising at the arch of the aorta, were completely surrounded and compressed by the new growth. The trachea and its main bronchi were embedded in a firm mass of white newgrowth. The walls of the pulmonary arteries were, at the hilum of the lung, infiltrated with tumor. The inferior vena cava, as it entered the heart, was surrounded by newgrowths, which extended into the substance of the right auricle and lined its cavity almost completely. The tricuspid valve had some of the newgrowth infiltrating its leaflets from the right auricle. The pulmonary valves were free, although their bases rested upon infiltrated tissue. The same was true of the aortic and mitral cusps.

Aorta. The wall was thin, with a few fatty plaques in the intima posteriorly. The glands about the aorta in the abdomen were œdematous, as was the rest of the tissue. The glands were, otherwise, not enlarged, nor showed tumor.

Abdomen. The peritoneum was clear and shiny. There were some few old adhesions between the gall-bladder and the transverse colon. The peritoneum contained over 750 c.c. of a yellowish and milky-looking fluid.

Œsophagus. Organ was clear throughout. There was no evidence of pressure from tumor in any direction. The tumor mass in the thorax lay anterior to the œsophagus.

Stomach. Contracted and congested throughout. The mucous membrane was clear and velvety. There was no sign of tumor mass in its walls. The duodenum was also congested. Mucosa was bile-stained.

Intestines. There was some congestion and œdema of the intestines, otherwise there was no change. The large intestine had the same characters as the small bowel. The appendix was free. The mesentery of the small intestine was œdematous, and, when cut into, exuded a milky fluid.

Rectum. Clear.

Liver. 1920 grams, 26 x 19 x 8 cm. Organ was of good size; capsule was smooth and tense; organ congested. There was œdema of the gall-bladder, which contained a blood-stained looking bile. The inferior vena cava, as it passed behind the liver, was encircled by tumor mass, which also infiltrated the openings of the hepatic vein. Between the suspensory ligaments the tumor mass lay beneath the capsule of the liver, and infiltrated the liver substance itself. On section, the tumor was found to infiltrate the upper border of the liver, which lay against the diaphragm, for a depth of 4 cm. Liver substance in this area was completely obliterated and looked like bone marrow. Save for this continuous infiltration, the liver contained no other tumor masses. The left lobe of the liver was free. The liver substance itself had a nutmeg character.

Pancreas. 18 cm. long. Organ was pinkish-gray in color; lobules were distinct; the organ was healthy looking.

Spleen. 185 grams, 12 x 4 x 3 cm. Organ was dark in color, very firm, and of cardiac nature; otherwise it showed no changes. The mesenteric glands were slightly enlarged and œdematous.

Adrenals. The left adrenal was healthy; organ was rather dark in color, but showed no change in substance. The right adrenal lay close under the diaphragm of the right side, and there was a continuous infiltration of tumor mass from the infiltrated diaphragm over the peritoneal surface of the adrenal and kidney. The right adrenal showed a small nodule, 1 x 1.5 cm., with tumorous substance within it.

Left Kidney. 200 grams; organ was firm and œdematous, 12 x 3.75 x 5 cm. Capsule was thin and peeled readily. Organ was indurated. The cortex was swollen. A slight amount of tissue was removed with the capsule, near the hilum. The pelvis and ureter were free.

Right Kidney. 230 grams, 12.5 x 3.7 x 5 x 4 cm. Organ showed a tumor mass beneath the capsule, 6 mm. in diameter. The kidney substance was infiltrated as far as the pyramids and about the pelvis. The tumor mass was light in color and, on the surface, displaced kidney tissue entirely. Ureter was free. The spermatic vein was thrombosed. The inferior vena cava opposite the renal vein showed a large and firm thrombus, firmly adherent, measuring about 4 cm. in length. Its lumen, however, was not entirely obliterated.

Bladder. Several veins about the bladder in front, and also the prostatic plexus, were thrombosed. The bladder mucosa was reddened and slightly œdematous, but otherwise healthy.

Prostate. Normal. Testes were clear.

Bones. Sternum: Opposite the tumor mass of the right thorax the sternum was infiltrated in its bony and cartilaginous tissues, as well as the intercostal muscle, by newgrowth. The bone was much softened, and, in parts, appeared carious. The plaque of infiltration of newgrowth against the sternum was 11 cm. in diameter, from above downward, and lay mostly to the right side. Opposite the clavicles the tumor lay on the first rib and was adherent to it on the left side. The right side was free. The vertebræ did not appear infiltrated.

Microscopic Examination. Aorta. The aortic wall, including intima and media, was clear, save for the faintest fatty degeneration of some of the muscle cells. Immediately beyond the media, that is, in the adventitia, the aorta was nothing but tumor, with some bloodvessels within it, The tumor cells were mainly of the small round-cell character, with a few spindly-looking cells and nuclei mixed in it. It was remarkable that no tumor infiltration was to be found in the media of the aorta, although the tumor cells came directly up to this tissue. The tumor cells were the size and shape of lymphocytes, with relatively large and dark-staining nuclei, with but little protoplasm surrounding them. The cells were massed together, with little stroma between them, nor was there any capsule or membrane limiting the tumor mass. The tumor cells advanced at the periphery by infiltration.

Heart. The heart muscle, where such was present, appeared healthy, save that in places it had suffered from compression. The pericardial surface showed a layer of fibrous tissue on the outside, with an infiltration of tumor cells beneath it. The epicardial fat was infiltrated with tumor tissue. All through the heart muscle, and lying between the bundles of muscle cells, was tumor tissue of the character of a small round-cell sarcoma. The normal fibrous tissue of the heart was still to be made out. In one area tumor cells were to be found within a bloodvessel. The infiltration of tumor was frequently in large heavy strands.

A transverse section was taken of the ventricular septum on the right side just below the insertion of the tricuspid valve. The section extended across the entire septum through the regions occupied by the bundle of His, and extended half way through the thickness of the septum, which, at this part, varied up to 2 cm. in diameter. Macroscopically, the septum, at this point, showed only a few heart muscle fibers at the extreme ends (right and left) of the septum. In the sections made it was found that sarcomatous tissue had almost entirely displaced the heart muscle, and only here and there were some bundles of fibers, whose individual elements were permeated with newgrowth. In none of the sections could the bundles of His be distinguished, or any trace of them found.

The entire interventricular septum was obtained, special precautions being taken to preserve the septum fibrosum and the surrounding tissue. The septum was then divided into blocks suitable for section, and serial sections were made from before backward. In this way the entire thickness of the septum was obtained, and the entire area covered by the bundle of His was examined.

Although the course of the auriculo-ventricular bundle has been fairly well defined, and the situation of the two limbs in the two ventricles of the normal heart is known, yet considerable difficulty is experienced in definitely locating the position of the muscle bundles in a specimen so completely disorganized by newgrowth as the one under observation.

As above stated, the greater portion of the interventricular septum is infiltrated with newgrowth to within a short distance of the apex. The infiltration of the new growth into the septum has thickened it and increased its bulk to a considerable extent. The infiltration is greatest at the upper portion of the septum, and occupies its tissue from its anterior to its posterior margins. The endocardial surface appears quite smooth, and the endocardium seems to be retained over it. On cutting into the septum, one cannot distinguish the presence of muscle tissue with the naked eye. The white and infiltrated appearance of the septum is the same from both ventricles.

The sections of the septum show the muscle tissue almost entirely replaced by tumor cells. These latter cells are of the small round variety, having in the most part a round, darkly-staining nucleus, with very little protoplasm surrounding it. The cells take no definite arrangement among themselves or at the stroma. The stroma is very sparse and appears to be that which normally exists in the heart muscle. The tumor advances at its borders by infiltrating the connective tissue between the muscle fibers and by their mass, obliterating the muscle cells of the part. The infiltration of the tumor beneath the endocardium appears more rapid than in the muscle tissue. The most advanced infiltration is found immediately beneath the endocardium and its connective tissue layer. The degree of infiltration of the musculature varies in different parts, but in general the greatest infiltration and obliteration of muscle tissue is found in the uppermost part of the septum.

The usually thin and tendinous septum fibrosum is half an inch in thickness, being occupied over its right surface by a thick tumor mass. This extends downward on the right side beneath the endocardium.

Along the anterior and lower margin of the septum fibrosum, where connective tissue strands pass in a V-shaped figure into both ventricles, there is considerable tumor infiltration, but there remains, however, a certain number of muscle fibers at the point of the fibrous division. These fibers prove to be the remains of the heart muscle and distinctly show transverse striations of their substance. Directly beneath the endocardium of the left ventricle tumor cells occupy almost the entire tissue, leaving only here and there occasional strands of connective and elastic tissne, while among these an occasional cell, looking like a muscle fiber, is encountered. These apparent muscle fibers are considerably distorted by the pressure of the tumor cells. Close examination does not reveal any cross striations. Their position is usually among what are connective tissue strands beneath the endocardium, and occasionally elastic fibers are demonstrated in their neighborhood. Although serial sections were obtained of this area, these fibers cannot be followed as a bundle for any distance, as these elements exist only as scattered cells, having, histologically, no close association with other fibers like them.

In the sections made anterior to the septum fibrosum, no elements of this nature were apparent.

Again, in sections made transversely through the septum at various levels, definite muscle elements, occupying the position of the bundle of His, could not be made out more clearly than the fibers already described. Everywhere these fibers appeared atrophied, and had lost their usual plumpness. At times even the fresher of the surrounding tumor cells compress the individual fibers. No muscle elements having even a suspicion of the His' bundle are evident in the right ventricle.

Lung. The alveoli were still well marked out, but were filled with a clear exudate and red blood cells. There was no leukocytic infiltration.

Spleen. Fairly congested, and lymphoid tissue was rather diffuse. There was no evidence of tumor infiltration.

Adrenal. Section consisted almost entirely of tumor tissue, with strands of adrenal cortex scattered through it. The staining differentiated very well the tumor from the adrenal. The tumor cells varied in their appearance from large protoplasmic cells to small, almost lymphoid, tissue; the medulla of adrenal did not appear infiltrated.

Kidney. The greater portion of the section was infiltrated with tumor mass. In infiltrated areas nothing remained of the tubules or of the original kidney substance, save the bloodvessels and glomeruli. The tumor mass was made up of irregular small cells, distributed in strands and small masses. There was a considerable stroma between the individual cells and masses of cells. In many cases the tumor cells were definitely spindle-shaped.

Liver. Very little was left of liver tissue in the section. Its entire area was occupied by tumor of a sarcomatous nature. Some of the original fibrous tissue bands were still present. Within the remaining liver tissue stagnated bile pigment was found. There was considerable stroma to be found in the tumor tissue.

To sum up, the anatomical findings which interest us most are the extensive sarcomatous infiltrations of the heart, its pericardium, and its large veins. In substance the heart is occupied by sarcomatous tissue in two-thirds of its mass. The sarcomatous invasion has replaced the heart muscle to a great extent, not only in its outer walls, but also in the auricular and ventricular septa. Histological examination of the septum demonstrates a disorganization of the bundle of His. Although we cannot definitely say that the bundle of His has been completely obliterated, yet truly recognizable

Purkinje fibers were not found. If we allow, however, that the distorted muscle-like elements appearing beneath and among the connective tissue fibers of the septal endocardium are elements of the bundle of His, we still find that these elements have been greatly reduced in number, and those that are present have been compressed and distorted by the sarcomatous infiltration. It is not at all clear to us that all these subendothelial fibers are of the Purkinje type. In fact, we must admit that some are, most probably, elements belonging to the longitudinally disposed heart muscle fibers, which appear superficial to the situation of His's bundle.

In view of the extensive disorganization of the bundle of His, it is difficult to correlate the clinical findings with the generally accepted views concerning this bundle. If the bundle of His is alone responsible for the maintenance of the proper rhythmic contraction of the ventricle, then we must assume that an extremely small number of these fibers can carry on that function for the proper maintenance of life. In the case described, the few Purkinje fibers which remained (if such they were), were very much altered by sarcomatous invasion and pressure. The function of these few cells must have been considerably diminished, and could only have supplied impulses to a small portion of the ventricle.

It is more than likely that the heart is dependent not alone upon the bundle of His for the transmission of impulses and the proper correlation between the auricle and ventricle, but that other factors, such as the cardiac muscle, central nerve fibers, and cardiac ganglia with their nerve plexuses, each and all may assume a portion of the work of maintaining a proper control and relationship of auricle and ventricle.

THE REACTION OF VARIOUS BACTERIA UPON AESCULIN AGAR.*

OSKAR KLOTZ AND A. C. RANKIN.
(From the Pathological Laboratory of the Royal Victoria Hospital, Montreal.)

GREAT attention has been given to the bacteriological analyses of "open waters" to determine their value as potable waters. Exhaustive determinations have been made of the bacterial flora of various rivers and lakes. The common result of most of these observations has been that the colon bacillus is present to a greater or lesser degree in every "open water." However, tho different streams may contain colon bacilli, it is found that the numbers which are present in a definite quantity of the water vary; but when a certain stream is analyzed at intermittent periods for a year or more, it is found that the number of bacilli present in that water is fairly constant for the same period in the year, except when a new source of pollution has entered the stream within the viable distance of the colon bacillus. If this source of pollution is continuous, a new colon standard is developed for that stream.

It is universally accepted that B. coli is a fair indicator of pollution in any water, and that when the "normal" coli content has been determined in a water, any unusual fluctuation, arising above this normal, is indicative of a new pollution.

Hence, too, a bacteriological analysis of water mainly concerns itself in the determination of the presence of colon bacilli, besides estimating the total bacterial count per cubic centimeter of water, and the number of liquefiers of gelatin present. When occasion demands, definite pathogenic organisms are searched for in the water.

As, therefore, the routine water analysis is so closely associated with colon bacilli, many investigators have devised special methods to determine the colon bacillus in definite quantities of water. Most of these methods have been, in truth, devices for separating the colon bacillus from the many other organisms present in the water, and some workers have obtained a definite biological reaction on certain media to serve as an indicator of the presence of this bacillus.

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It has been found that the colon bacillus is more resistant to certain chemical substances than are other bacteria, so that media containing these substances will propagate this bacillus better than others. Such media have been prepared with phenol and hydrochloric acid (Parietti), crystal violet (Conradi-Drigalski), malachite green (Löffler), bile (McConkey), caffein, and other substances. By the use of these media most of the bacteria other than those belonging to the colon-typhoid are group inhibited in their growth. From such cultures then the remaining bacteria may be isolated and their characters determined. Some workers deem it quite sufficient to inoculate dextrose fermentation tubes from growths in the above inhibitory media, and to determine (by the production or non-production of gas in the proportion of $H:CO_2=2:1$) the presence or absence of the colon group. This presumptive test is used extensively in America. In the English laboratories the use of the bile-salt media has found preference (McConkey).

It is scarcely necessary to point out that most of the presumptive tests have their shortcomings in one way or another. We have been particularly struck with the inaccuracy of the gas determination for the colon bacillus. We have noted that when colon bacilli were present in mixt cultures in the carbohydrate broth either they produced no gas whatever, or that the amount of gas was very much reduced, and in altered proportions.

Recently Harrison and van der Leck[1] have introduced still another medium for the determination of colon bacilli and excretal bacteria in water or milk. They have found that when the glucoside (aesculin) is present with iron citrate in the culture medium, the colonies of colon bacilli are black and have a black halo around them. These black colonies are readily distinguishable against a white background. They have found that B. lactis aerogenes, lactose-fermenting yeasts, and some molds give the same blackening of the media about the colonies as do colon bacilli. As, however, the yeasts and molds are readily distinguished in their colonies and are seldom found in water, they may be disregarded. In some of the experiments Harrison and van der Leck found that the colonies of B. lactis aerogenes could be distinguished from those of the colon bacilli. They point out that as the B. lactis aerogenes may be regarded as an excretal form of organism, it is of value to recognize its presence. The authors state that some 40 species or varieties of bacteria and yeasts have been grown in media containing aesculin with negative results.

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[1] *Centralbl. f. Bakt.*, Abt. II, 1909, 22, p. 547; *Proc. Roy. Soc. Can.*, 1908, S. III, 2, p. 105.

The authors have prepared a bile-salt aesculin broth and an aesculin agar which they recommend for the routine examination of water. In using the former medium the presence of colon bacilli is indicated by the black coloration of the fluid, and they consider this a better presumptive test than the use of the neutral red bile-salt broth.

The authors have found that in the analysis of water, the blackened bile-salt aesculin broth has invariably yielded colon bacilli. In the analyses of 60 samples of water, they have found the aesculin bile-salt test correct in 100 per cent.

In the following experiments we have undertaken the examination of many strains of colon bacilli, which have been isolated and fully determined as to their characters, besides testing the action of other bacteria on the aesculin media. Most of the bacteria tested were obtained from patients in the Royal Victoria Hospital. These bacteria had been isolated from time to time, and were retested as to their purity on several occasions.

In determining the action of the bacteria on aesculin media, we have used the formula as given by Harrison and van der Leck. The method of preparing the agar is as follows:

1 or 2 gm. of Witte's peptone.
0.5 gm. sodium taurocholate (commercial).
0.1 gm. aesculin.
0.05 gm. iron citrate (ferric).
100 c.c. tap water.

After steaming 15 to 30 minutes the medium is filtered and sterilized. For aesculin agar 1.5 per cent agar is used, being dissolved in part of the water, after which the other ingredients are added, the medium brought to boiling, filtered, tubed, and sterilized.

Plates were poured with this medium, and after hardening, the different organisms were planted on the surface, or deep colonies were made. The plates were incubated at 37° C. and were examined every 24 hours for a period of two weeks.

In most instances in which a positive result was obtained, the discoloration of the medium occurred within 24 hours. In a few instances, however, the positive result was delayed for several days. One of the colon strains gave a positive result on the second day. Another colon strain was positive only on the third day, while a strain of B. mucosus remained negative until the fifth day, when a slight blackening of the medium occurred, which increased for several days. We have found that there is a certain variation in the positive tests. The typical positive tests in which the colonies are black with a black halo around them, as described by Harrison and van der Leck, do not constantly occur. Four colon strains we found gave only a faint brownish discoloration of the medium, immediately about the colony, while the colony itself was of a greyish-brown color. Again other colon colonies gave a reddish-brown discoloration of the medium without definite blackening of either the colony or the medium. One strain of proteus, which gave a positive result, first developed a yellow change in the medium, which later showed a greyish halo around the outer edge of the discoloration.

On account of the variable types of the color reactions which were obtained on the medium, we have included in the "positive tests" all reactions which in any way discolored the medium. This discoloration ranged, as above noted, from a yellowish-brown to a distinct black. The tests referred to as negative were those in which no color was present in either the colony or the medium. In this way we have tested 110 organisms of different strains, and from various sources with the following result:

	Positive Test on Aesculin Agar	Negative Test on Aesculin Agar
B. coli communior	3	10
B. coli communis	10	6
B. typhosus	0	19
B. typhosus-like organism	1	0
B. paratyphosus B	0	13
B. mucosus	3	1
B. duodenale (Ford)	1	2
B. lactis aerogenes	3	1
B. pseudodysenteriae (Muller)	1	1
B. dysenteriae (Flexner)	0	1
B. acidiformans	0	1
B. alkaligenes	0	2
B. iliacus	1	2
B. cloacae	1	4
B. subcloacae	1	0
B. proteus vulgaris	1	9
B. proteus plebeius (Ford)	0	1
B. pylori (Ford)	0	1
B. xerosis	0	1
B. subtilis	0	1
B. diphtheriae	0	3
Staph. aureus	0	2
Staph. albus	0	3

It will be seen from the above table that most variable results were obtained from the various organisms. B. coli communior gave only three positive tests in 13 strains, while B. coli communis gave 10 positive reactions in 16 strains. The positive tests obtained with the B. coli communis and communior were far from being uniform, and the color reactions varied from those producing no color at all, others producing a faint greyish-brown, to those showing red-brown, brown, or black color. These color reactions were evident either in the colonies themselves, or in the surrounding medium, or in both. The majority of the colon strains which gave a color reaction showed the dark punctate center in the colony with a black halo surrounding the colony, as described by Harrison and van der Leck.

Among our stock of 19 typhoid strains none was found to give any color reaction on aesculin agar. We have, however, an organism which was isolated from the gall-bladder of a typhoid subject, and which on media gave all the reactions of B. typhosus. At the time of isolation, this organism was tested with a known typhoid serum but gave a negative result. Subsequently at intervals of about a month this organism has been tested with various typhoid sera and has never agglutinated in dilutions higher than 1.25. Except for the agglutination reaction, this bacillus has all the features of B. typhosus. This bacillus was found to give a positive reaction on aesculin and the reaction was positive only on the seventh day, no reaction showing in the six days previously. The medium about the colony was discolored a faint brown which increased to a darker brown after several days. The reaction here obtained, altho not typically positive, as described by Harrison and van der Leck for certain colon strains, was nevertheless equal in intensity to that obtained with several colon organisms tested by us, and noted as positive in the above table.

Again, among four B. lactis aerogenes strains, we had one which remained negative in its reaction on aesculin agar after 14 days.

Summarizing our results, we found that in 62 different strains of 13 types of intestinal bacteria of man, 22 gave a positive reaction on an aesculin, while 40 were negative. In other words, the majority of the excretal type of bacteria, which were examined by us, and which had their known origin from man, gave a negative result when tested on the above medium.

In our hands this medium has not proved satisfactory for the isolation and determination of B. coli and other human excretal bacteria.

NOTE.—Since going to press Harrison and van der Leck have in a recent article (*Centralbl. f. Bact.*, 1909, 51, p. 607) described some modification in the preparation of the media. The amount of iron citrate has been increased to 0.1 per cent, and they insist that the acidity of the media should be 0.6 per cent acid. The media which we have used in our experiments was of this acidity, but contained a smaller quantity of iron citrate. The authors point out that with an acidity lower than 0.6 per cent the results are not uniform. It is found too that the color of the colonies is dependent directly upon the amount of citrate and acid present.

THE CYTOLOGY OF CHRONIC MIDDLE-EAR DISCHARGES.*

BY E. HAMILTON WHITE, M. D., AND OSKAR KLOTZ, M. D., MONTREAL.

The importance clinically of a chronic discharge from the middle ear has long been recognized. The fatality of the intracranial complications, septic meningitis, extradural abscess, brain abscess, or sinus thrombosis, which may originate in such a condition, must impress all with the desirability of preventive treatment. Usually by the time indications of intracranial involvement are present the chances of saving the patient are slight, although modern surgery has done wonders towards improving the outlook even at this late stage.

On the other hand it is well recognized that the large majority of such cases go through life from the cradle to the grave without much inconvenience apart from impaired hearing and what little attention is required by the discharge.

The present investigations were undertaken to see if by an examination of the cytological features of these discharges we could find any constant characteristics by which such cases could be segregated into groups, which would help in making a prognosis as to the probable course of the disease, or would give any reliable guide for the management of the case, especially as to when radical operative interference should be advised.

The origin of most cases of chronic suppurative otitis is apt to be obscure. The ear is usually allowed to run for two or three years before an otologist is consulted. A great many cases start in early childhood, so that the origin is unnoted or forgotten. From what we know of the pathology of the condition it would seem that the stamp of chronicity is usually received at the original onset of the trouble. By this we mean that the destructive processes have gone so far that a complete repair even approaching the normal cannot occur. A cure in the sense of a "restitutio ad integrum" is out of the question, quiescence with absence of active symptoms is the best that can be attained.

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AUTHOR'S NOTE: The claim has been made by Dr. Milligan,[1] in an address before the Otological section of the British Medical Association that by determining the cytology of middle ear discharges he could differentiate various types of otitis and fix indications for radical operative interference. A criticism of his paper by one of us, (O. K.),[2] called forth a reply from Dr. Milligan[3] defending his views as originally expressed. It was of interest to find in this reply that his claim to have found giant cells in the "exudate" of cases of tuberculous otitis was based on the examination of material collected by a curette, in other words tissue definitely not in the exudate. As we are unable to agree with Dr. Milligan either on pathological or clinical grounds we submit the following results of examined cases where careful percentage counts of the cells present were made.

From a clinical standpoint cases may be divided into groups according to the degree of local destruction and probable cause of the chronicity of the discharge.

In a large group of cases the determining factor of chronicity appears to be a permanent perforation of the drum-membrane, which allows secretion from the Eustachian tube to flow into the middle ear. This sets up more or less irritation or inflammation of the middle ear itself, which may be: (1) limited to the region of the tube, (the hypotympanum), these cases we have classified as due to chronic Eustachian tube catarrh; or (2), it may involve all the middle-ear spaces, these are classified as due to chronic Eustachian tube catarrh, with chronic inflammatory changes in the middle ear itself.

In such cases the degree of disease in the Eustachian tube and nasopharynx is of course a factor of the highest importance in determining the amount of discharge, and to some extent the degree of secondary change in the middle ear.

In the third group the chronicity is determined by the severity of the destructive process in the middle ear, and the character and degree of the reparative process and is more or less independent of the presence or degree of disease in the Eustachian tube and nasopharynx, although such disease may be a very important contributory cause of the chronicity. The most important cases in this group are those complicated by the development of cholesteatoma. Where such a classification can be made it is a most important step in determining the course of treatment best calculated to arrest the process and to bring the ear to a condition of quiescence. There are always two objects to be attained in the treatment of a case of chronic middle-ear suppuration: (1), to insure the safety of the patient against any possible danger of intracranial involvements, (this is, of course, imperative, and before it all other considerations must fade into insignificance), and (2), to arrest the discharge, if possible; this is of more or less relative importance.

Cases included in the first group are to be regarded as offering practically no danger of intracranial involvement, and may, therefore, be indefinitely safely treated by the most conservative methods. Cases of the other two groups offer such a wide range of differences that the course of treatment must always be determined from the features of the individual case, and, although on general lines the indications of danger are well recognized, it must always be a matter for the judgment and experience of the surgeon in charge. The social condition of the patient is often of importance in deciding what form of treatment to advise. If the means and opportunity for competent conservative treatment are available radical operation may often be avoided or indefinitely postponed, whereas if without opportunity to get competent attention it is safer for such a patient to have a radical operation.

The mucosa of the middle-ear spaces, except in the region of the Eustachian tube, consists of a single layer of flattened epithelium lying on a small amount of connective tissue, which separates it from the bone and carries the blood supply, it is really a mucoperiosteum. The epithelium is of mucosal origin and not at all similar to the squamous epithelium of the skin surfaces. The epithelium of the Eustachian tube and its immediate neighborhood is of the ciliated columnar type, and in the transition from this to the flattened epithelium of the middle ear there are found ciliated cells of the cubical and flattened type. In the new-born the amount of submucosal connective tissue is quite considerable, but this is normally resorbed as the individual develops so that in adult life it is very slight. This is incidentally an important factor in determining the course and results of middle-ear inflammation in infancy. It is generally held that there are no mucous glands in the mucosa of the middle ear,[4] though Politzer[5] states that he has observed them ocasionally in the region of the Eustachian tube. In the tube itself they occur abundantly.

The inflammatory changes in the mucosa of the middle ear consist in mild cases of swelling of the submucous layer with engorgement of the blood vessels and more or less desquamation of the epithelium. If the inflammation subsides promptly the areas denuded by desquamation are recovered by regeneration of the mucosa and a return to a normal histological picture occurs. Where the inflammation is prolonged, proliferation of the submucosa occurs and a polypoidal condition is produced. In such a change in the mucosa we can find both a direct and indirect cause for chronicity of the ear inflammation. In the first place such a mucous membrane can rarely return to a healthy state, and, secondly, the thickening, especially if it occur in the region of the auditus involving the folds about the ossicles, tends to cause more or less retention of the exudate, or discharge, and thus to delay or prevent healing. Where the inflammatory process is more severe, extensive denudation and ulceration occur, which may involve the underlying bone. Repair of this extensive denudation is often accomplished by inward growth of the epidermis from the external auditory meatus. It may sometimes occur from the remains of the mucosa, but in such a case the cells of the mucosa tend to show a metaplasia to the epidermal type. It is the epidermisation of the middle-ear spaces which forms the basis of the cholesteatomatous condition frequently met with in middle-ear suppuration. It is now recognized that the destructive characters of cholesteatoma arise purely from the retention of inflammatory productions and that the pearly masses sometimes found are only the products of desquamation of the epidermis which has invaded the middle-ear spaces.

From this brief outline it will be seen that the pathological conditions in a case of chronic suppurative otitis media are apt to be extremely complicated. From the presence of secondary infection, which is, we may assume, constantly present there is to be expected a more or less active reaction to infection, while in certain regions the disease has reached a stage of extreme chronicity or is healed.

From our knowledge of the histology we know that mucus in any considerable amount must come from the Eustachian tube, whatever other portion of the middle ear is involved. Where the middle ear is inflamed without tube involvement, as in cases of attic disease, the discharge is always of purulent or seropurulent nature with scanty mucus.

The technique of collecting the smears was as follows: The ear was thoroughly cleansed by syringing and careful swabbing. Where there was evidence of disease in the region of the attic or antrum the cleansing was extended to these regions by an intratympanic irrigation by means of a Hartman's cannula. The ear was then left without treatment for twenty-four hours, being protected by a piece of sterile wool in the meatus. Where discharge was abundant the excess was swabbed out of the meatus before collecting the smear, but in several cases very little discharge was present and, therefore, it had to be collected directly by introducing a fine swab through the perforation. All possible care was taken to reduce possible contamination from the meatus to a minimum.

Several smears were carefully made from each case and were dried and fixed. Several staining methods were usually employed.

In most instances one specimen was stained with carbolthionin and another with Wright's stain. Occasionally, too, specimens were stained with weak fuchsin, eosin or Gram.

The specimens were examined at the laboratory without having any information as to the nature of the condition in the ear. Cell counts were made from each. In many instances there was some difficulty in counting the cells, as many of the leucocytes and other cells had undergone degeneration, so that their nature could not be determined definitely. Hence in the counts given below only those cells are included in which the characters were quite definite. It is not an uncommon finding in exudates to have portions of fragmented nuclei lying amidst the debris of the exudate and at times much care must be exercised to differentiate some of these from very small lymphocytes. The squamous epithelial cells present the least difficulty of recognition. In no instance did we find coarsely granular eosinophiles, though polymorphonuclear leucocytes with acidophilic cytoplasm were encountered.

The cell counts as they were obtained in this series of cases are given in the following classified list:

GROUP I.

Clinically grouped as due to chronic Eustachian tube catarrh.

Case 1: Polymorphonuclear, 88 per cent; Lymphocytes, 4 per cent; Large Mononuclear, 3 per cent; Endothelial, 5 per cent; Epithelial, 0 per cent.

Large numbers of bacteria, both bacilli and cocci, were seen. The bacilli appeared to be of various kinds. The leucocytes were surrounded by a considerable amount of mucus in strands. The leucocytes showed degenerative changes.

Case 5: Polymorphonuclear, 95 per cent; Lymphocytes, 1 per cent; large Mononuclear, 1 per cent; Endothelial, 1 per cent; Epithelial, 2 per cent.

Moderate amount of mucous Leucocytes well preserved. Fair number of diplococci looking like pneumococci.

Case 8: Polymorphonuclear, 88 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 0 per cent; Endothelial, 10 per cent; Epithelial, 1 per cent.

There was a fair amount of mucus. A few bacteria were seen and there was some debris.

Case 9: Polymorphonuclear, 94 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 0 per cent; Endothelial, 4 per cent; Epithelial, 1 per cent. There was a moderate amount of mucus in the smear; some debris and a few bacteria.

Case 10: Polymorphonuclear, 94 per cent; Lymphocytes, 3 per cent; Large Mononuclear, 0 per cent; Endothelial, 2 per cent; Epithelial, 1 per cent. There was much mucus; very few bacteria.

Case 13: Polymorphonuclear, 81 per cent; Lymphocytes, 0 per cent; Large Mononuclear, 5 per cent; Endothelial, 14 per cent; Epithelial, 0 per cent. There was no mucus present. There were many degenerate cells and debris seen in the stained smear. A fair number of organisms, mostly diplococci, were present.

Case 14: Polymorphonuclear, 59 per cent; Lymphocytes, 26 per cent; Large Mononuclear, 7 per cent; Endothelial, 8 per cent; Epithelial, 0 per cent. There was a considerable amount of thick secretion with some mucous strands in the smear. Bacteria were fairly abundant, there being both bacilli and cocci present. Many of the cells showed fragmentation with liberation of nuclear masses from the protoplasm. A few red corpuscles were also present.

Case 16: Polymorphonuclear, 89 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 1 per cent; Endothelial, 9 per cent: Epithelial, 0 per cent. There was very little mucus present in the smear. The cells were well preserved. There were very few bacteria present.

Case 19: Polymorphonuclear, 93.5 per cent; Lymphocytes, 3 per cent; Large Mononuclear, 1 per cent; Endothelial, 2.5 per cent: Epithelial, 0 per cent. There were very few strains of mucus. The secretion between the cells was otherwise thin. There were large numbers of diplococci throughout the smear. The cells stained well Columnar epithelial cells could not be recognized.

GROUP II.

Classified as due to chronic Eustachian tube catarrh, with secondary chronic inflammatory changes in the middle ear.

Case 2: Polymorphonuclear, 97 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 0 per cent; Endothelial, 2 per cent; Epithelial, 0 per cent. The leucocytes were well preserved, and there was a moderate amount of mucus. Bacteria were scarce.

Case 4: Polymorphonuclear, 96 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 1 per cent; Endothelial, 2 per cent; Epithelial, 0 per cent. There was a small amount of mucus. The leucocytes were well preserved. There were fair numbers of diplococci looking like pneumococci. No other bacteria were present

Case 20: Polymorphonuclear, 86 per cent; Lymphocytes, 3 per cent; Large Mononuclear, 1 per cent; Endothelial, 9 per cent; Epithelial, 1 per cent. There was little or no mucous present, and bacteria were almost wanting, occasional diploccocci being seen.

GROUP III.

Classified as showing extensive histological changes in the middle ear (Cholesteatoma, etc.)

Case 3: Polymorphonuclear, 85 per cent; Lymphocytes, 0 per cent; Large Mononuclear, 0 per cent; Endothelial, 1 per cent; Epithelial, 14 per cent. No mucus was present. There were innumerable bacteria of various kinds, including Vincent's fusiform bacillus and a spirochaete. Many of the squamous epithelial cells were devoid of nuclei. There was some debris.

Case 11: Polymorphonuclear, 85 per cent; Lymphocytes, 0 per cent; Large Mononuclear, 0 per cent; Endothelial, 0 per cent; Epithelial, 15 per cent. Some mucus and debris were present. There was a very large number of bacteria.

Case 12: Polymorphonuclear, 16 per cent; Small Lymphocyte, 6 per cent; Large Lymphocyte, 0 per cent; Endothelial, 0 per cent; Epithelial, 78 per cent. There was little mucus and some debris. No bacteria were present.

Case 15: Polymorphonuclear, 98 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 1 per cent; Endothelial, 0 per cent; Epithelial, 0 per cent. There was little or no mucus present. The pus cells were well preserved, and the protoplasm had acidophilic characters. Several polynuclear eosinophiles were found. There were numerous bacteria present, most of which were large bacillary forms.

Case 17: Polymorphonuclear, 94 per cent; Lymphocytes, 1 per per cent; Large Mononuclear, 1 per cent; Endothelial, 0 per cent: Epithelial, 4 per cent. The protoplasm of the polynuclear cells was finely granular and pink staining. There was a slight amount of mucus present. Bacteria were infrequent.

Case 18: Polymorphonuclear, 72 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 1.5 per cent; Endothelial, 3 per cent; Epithelial, 22.5 per cent. There was a great deal of mucus in which the cells of the exudate were embedded. The epithelial cells were scattered sometimes singly, and at other times in small plaques. These epithelial cells were at times without nuclei and formed thus homogenous "Squamae," not taking the basic stains.

Case 21: Polymorphonuclear, 82 per cent; Lymphocytes, 4 per cent; Large Mononuclear, 0 per cent; Endothelial, 5 per cent; Epithelial, 9 per cent. There were some strands of mucus throughout the smear. There were innumerable bacteria of various kinds present.

Case 22: Polymorphonuclear, 98 per cent; Lymphocytes, 0 per cent; Large Mononuclear, 0 per cent; Endothelial, 1 per cent; Epithelial, 1 per cent. The cells were not very numerous. There were some strands of mucus between the cells of the exudate. Very few bacteria were seen.

ACUTE CASES.

Examined for Purposes of Comparison.

Case 6: Polymorphonuclear, 98 per cent; Lymphocytes, 2 per cent; Large Mononuclear, 0 per cent; Endothelial, 0 per cent; Epithelial, 0 per cent. There was a considerable debris, and some Gram-positive diplococci, some of which were in short chains. No mucus was present.

Case 7: Polymorphonuclear, 63 per cent; Lymphocytes, 3 per cent; Large Mononuclear, 3 per cent; Endothelial, 31 per cent; Epithelial, 0 per cent. There was a slight amount of mucus, and fair numbers of bacteria were present, mostly diplococci.

Case 23: Polymorphonuclear, 91 per cent; Lymphocytes, 2 per cent; Large Mononuclear, 1 per cent; Endothelial, 6 per cent; Epithelial, 0 per cent. There was very little mucus present. The cells stained well. There were some bacteria, both cocci and bacilli, found in the smear.

Case 24: Polymorphonuclear, 88 per cent; Lymphocytes, 5 per cent; Large Mononuclear, 0 per cent; Endothelial, 7 per cent; Epithelial, 0 per cent. There was no mucus in the secretion. There were large numbers of bacteria, both cocci and bacilli.

Case 25: Polymorphonuclear, 60 per cent; Lymphocytes, 1 per cent; Large Mononuclear, 15 per cent; Endothelial, 24 per cent; Epithelial, 0 per cent. There was little mucus in the secretion. Occasional chains of cocci were present.

(By endothelial cells we refer to the large Hyaline non-granular cells.)

As will be seen from the above examinations there is little uniformity in the cell counts of the different types. The one prominent feature which is noted is that the polymorphonuclear leucocytes are almost constantly in the greatest number. These cells predominate in the lesions occurring in the various parts of the ear and in the cases of varying intensity. In only one case did the lymphocytes appear in numbers up to 26 per cent, and this case had no particular features of chronicity. In fact, from the numbers of polymorphonuclear leucocytes and of lymphocytes we were not able to find any parallelism with the clinical features. On the other hand, the presence of epithelial cells in demonstrable numbers, was found only in cases of cholesteatoma. This is what we might expect from what we know of the disease.

The relative proportion of endothelial cells in the exudate did not give us any information as to the nature or progress of the case. It was first thought that their presence indicated chronically healing tissues, but this was not borne out when the cases were compiled. In none of the exudates examined by us have we been able to distinguish columnar epithelial cells as such.

In short, we are led to the conclusion that the cytological examination of ear exudates does not, save in the case of cholesteatoma, give any definite information as to the nature and site of the lesion, nor can the prognosis be foretold by this means.

One might say that practically in all cases of chronic suppurative otitis there is the predominance of polymorphonuclear cells. According to the contention of Dr. Milligan these should be regarded as acute cases and a favorable prognosis given.

Our experience has been that clinically some of the cases became quiescent, while others did not, according to the degree of success with which the condition of the Eustachian tube could be treated. In one case (Case II) a radical operation was performed on account of recurring attacks of pain and fever, but, although the ear spaces are now completely healed and the ear safe, there have been recurring periods of discharge from the Eustachian tube. I only one case (Case XIV) were the lymphocytes found present in considerable numbers. This is according to Dr. Milligan's statement an indication of chronicity and should be of weight in advising radical operative interference. This case was clinically grouped as one of tube catarrh and healed promptly after treatment of the nose and throat. It relapsed after two or three months, but quickly became quiescent as before. The only active symptoms in this case have been shown in small amounts of mucous discharge free from any offensive odor, which, however, have never been associated with pain or fever. In the few acute cases examined we can confirm Dr. Milligan's observation of the predominance of polymorphonuclear forms, but in view of our experience with the chronic cases this loses the interest which he would have us attach to it.

CLINICAL SYNOPSIS OF CASES EXAMINED.

Case No.	Age	Sex	Duration of Symptoms.	Probable Origin.	Amount and Character of Discharge.	Condition of other ear.	Remarks.
Group I.							Clinically due to chronic Eustachian tube catarrh.
I.	32	F.	2 years	Childhood	Small amount mucoid, no odor	M. T. perforated quiescent	Under treatment improved, but did not heal.
V.	20	F.	12 years	Scarlatina	Mod. amount mucoid, no odor	Case IV.	Under treatment improved, but did not heal.
VIII.	22	F.	19 years	Scarlatina	Mod. amount mucoid, no odor	Case IX.	Very persistent discharge without other symptoms.
IX.	22	F.	19 years	Scarlatina	Mod. amount mucoid, no odor	Case VIII.	Very persistent discharge without other symptoms.
X.	45	M.	6 weeks		Mod. amount mucoid, no odor	Normal	Became quiescent (old scars pointed to former otitis).
XIII.	18	M.	1 year		Mod. amount mucoid, no odor	Normal	Became quiescent, extensive destruction of M. T. (otitis in childhood?)
XIV.	28	F.	Indefinite	Childhood	Mod. amount mucoid, no odor	Normal	Became quiescent, relapses with small amount of discharge.
XVI.	58	M.	3 weeks		Small amount mucoid, no odor	Normal	Became rapidly quiescent (old perforation, childhood?)
XIX.	8	M.	6 weeks	Otitis in infancy.	Small amount mucoid, no odor.	Normal	Periodical discharge and quiescence.
Group II.							Classified as due to chronic Eustachian tube catarrh with secondary chronic inflammatory changes in middle ear.
II.	15	F.	8 years	Diphtheria	Profuse, muco-purulent, no odor	Normal	Repeated exacerbations with pain (radical mastoid operation).
IV.	20	F.	12 years	Scarlatina	Small amount mucoid, no odor	Case V.	Chronic hypertrophic change in mucosa of inner wall of tympanum.
XX.	49	F.	6 weeks		Mod. amount sero-purulent, no odor	Normal	Scar of simple mastoid operation, performed 6 years before. became quiescent.
Group III.							Classified as showing extensive histological changes in the middle ear (Cholesteatoma, etc.)
*III.	15	F.	Indefinite, about 2 years	Measles, at age 5 years	Profuse, purulent, foul odor	Normal	Cholesteatoma (radical mastoid operation).
XI.	15	M.	10 years	Measles	Mod. amount, foul odor	Case XII.	Cholesteatoma, still under observation.
XII.	15	M.	10 years	Measles		Case XI.	Cholesteatoma, still under observation.
XV.	14	M.	3 years		Mod. amount, slight odor	Normal	Granulations in middle ear (passed out of observation).
XVII.	26	M.	14 years	Typhoid Fever	Small amount, purulent, foul	Normal	Cholesteatoma (radical mastoid operation).
*XVIII.	17	F.	5 years	Measles	Mod. amount, purulent, foul	Persistent perforation	Cholesteatoma (radical mastoid operation).
XXI.	51	M.	48 years	Otitis in childhood	Small amount, foul odor	Case XXII.	Still under observation.
XXII.	51	M.	48 years	Otitis in childhood	Small amount, slight odor	Case XXI.	Scar over mastoid, abscess in childhood.
Acute Cases							Examined for purposes of comparison.
XXIII.	29	F.	6 weeks	Acute coryza	Small amount, no odor	Normal	Patient had been examined 2 months before when M. T. normal.

Cases from autopsy where acute otitis was incidental finding acute suppurative otitis media without perforation of M. T.

VI.	2	months; cause of death, (Acute suppurative otitis media?) no other gross lesion.	No tuberculosis.
VII.	1	month; cause of death, Acute otitis.	No tuberculosis.
XXIV.	1½	months; cause of death, Acute gastro-enteritis.	No tuberculosis.
XXV.	6	months; cause of death, Diphtheria.	No tuberculosis.

*Reported in detail, Montreal Medical Journal, December, 1909, P. 841.

A constant finding of interest was the presence of epithelium in the cases of cholesteatoma. We say of interest rather than of value for in such cases the diagnosis can usually be readily made by the macroscopic appearances of the discharge and frequently by its offensive odor. The epidermal flakes can usually be readily recognized macroscopically in the washings from the attic.

We believe, therefore, that the cytological examination of chronic middle-ear discharges cannot be regarded as having any value as a means of classifying or selecting cases for any particular form of treatment or in making a prognosis.

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THE DISTRIBUTION OF FAT IN THE LIVER

By JOHN McCRAE, M.B., M.R.C.P., and OSKAR KLOTZ, M.D.

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THE DISTRIBUTION OF FAT IN THE LIVER.[1]

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We have endeavored in the work upon which this paper is based to obtain some information relative to the fat which appears in the liver at death, as well as its nature and distribution. Fat which can be stained and recognized as such appears in a large percentage of all cases irrespective of age; in the one hundred cases comprised in this series, ninety-eight showed a recognizable amount of it; of twenty successive cases taken from our autopsy series, all showed it. In one hundred cases, fourteen times fat was present in extreme amount, a condition certainly pathological, for in thirteen of these where mention was made of the point, the macroscopic appearance was abnormal; thirty-two times it was considerable in quantity, which condition is probably pathological, for it is attended by an undue friability of the organ; often, too, the lobules are much less distinct than in the normal liver. In fifty-two cases, the fat was slight in amount, and twice it could not be demonstrated. These fifty-four cases are open to the interpretation that the fat is merely the expression of low vitality of the cell which is in some way prevented from carrying out completely its metabolism.

The cell may be likened to a mill which, with the usual number of workers, can handle a certain quantity of raw material, transform it into the finished product, without storing any of it on the premises. Let the mill be only half efficient by reason of half its employees being idle, and half its raw material is stored up on the premises. Or if the mill is suddenly supplied by one and a half times its usual raw material, one half of its daily income in raw material will be stored up. Thus the cell appears encumbered by fat when its working efficiency is lowered or when its fat supply is abnormally increased. We do not know whether the fat is the real raw material,

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or is an imperfectly finished product, but we assume that fat microscopically shown as such in the cell is material which in the normal liver cell would not appear; in other words, the normal cell-processes are accomplished with soluble materials, and these are ordinarily not to be demonstrated.

For the demonstration of the various types of fatty deposit in the liver, we have found Sudan III to be the most serviceable stain. Other methods, including staining by Nile blue sulphate, were also used, but we experienced some difficulty in interpreting our results from these sections. Although one of us (Klotz) has shown that *in vitro* the neutral fats, the fatty acids and the fatty acid soaps do give certain differences of color, yet by the use of Sudan III here no differentiation can be made with certainty between the neutral fats, fatty acids or oleic acid soaps, more particularly as these substances occur in mixtures.

From our observations we have been able to distinguish four types of fatty deposits in the lobules of the liver. Although these four types are readily distinguished, and are quite characteristic in themselves, we are by no means convinced that they differ as definitely in origin. These four types of fat deposit in the liver are as follows: (1) fat deposits in which globules alone are present in the lobule; (2) fat deposits in which granules alone are present; (3) fat deposits in which globules and granules are found in the same lobule; (4) fat deposits in which granules and globules are found in the same cell.

In the above classification we are laying considerable stress upon the *appearance* of the fat deposit in the liver. We are fully aware that a classification determined upon the physical characters of a substance is insecure, yet, as we shall point out later, these physical differences are associated with fairly definite differential staining properties.

Globules of fat may be of various sizes, ranging from minute spherical bodies to large masses occupying almost the entire liver cell. In some cells fat droplets of the smallest size are seen. These droplets are commonly aggregated towards one side of the cell, leaving the rest of the cytoplasm fairly free from fat droplets. As they increase in size, the droplets become fewer in number and the nucleus becomes slightly shifted to the cell wall, away from the fat deposit. This eccentric position of the nucleus is increased as the fatty materials accumulate until eventually the nucleus becomes flattened and elongated along one border of the cell. When smaller globules are present, the nucleus occupies a central position and shows no evidence of distortion. Gradually, however, when the fat droplets in the protoplasm coalesce, the nucleus is shifted eccentrically, and eventually occupies a position on the outer border of the large fat drop. The ectoplasm of the cell forms the boundary of the fat drop, while the protoplasm of the cell is reduced to a small area lying about the eccentric nucleus. Both the nucleus and the reduced protoplasm form a crescentic mass over the fat drop. It is, however, to be noted that the nucleus never becomes so compressed as to simulate that of the true fat cell. When thick sections are made, and the nucleus of the liver cell is observed in a vertical position, it has a round contour, and gives the impression that it is lying in the centre of the cell. When seen laterally, however, the nucleus appears oval with blunt and rounded ends.

Occasionally liver cells are completely distended with fat; the fat, however, is separated into several globules which have not coalesced. These globules seem to have between them a thin dividing membrane, which prevents their coalescence. When such cells are ruptured, the fatty contents flow together to form a single mass.

Granules, on the other hand, are readily distinguished from globules of the smallest size. In the case of globules, the intensity of the Sudan absorption increases with the size of the globule, the smallest globules appearing the lightest in color. Granules are uniformly of a brownish color, not unlike that seen in the granules of brown atrophy. The contour of the granules is moreover irregular, and commonly one can make out sharp corners and edges in their structure. Cells are frequently packed with these irregular granules, in which there appears to be no tendency to coalesce. The granules frequently take a concentric arrangement about the nucleus, but in other cases they are distributed diffusely, or in the periphery of the cell protoplasm. These granules we think to be evidence of degeneration.

In staining with Sudan III and hematoxylin, one can readily pick out the areas occupied by fat and distinguish the zone in the lobule which is affected. In the cases in which there is diffuse globular infiltration of fat in the lobule, it is difficult to make out the individual liver cells, as the knife drags a considerable amount of fat which blurs the section. In these cases of diffuse infiltration each liver cell is distended with the semifluid fat. In the older specimens crystals sometimes appear in these fat globules, probably crystals of neutral fats.

To sum up this question, we admit at the outset that we have no power of differentiating with certainty neutral fats from fatty acids or soaps. It is notable that fat appears in two forms, granular and globular, differing in appearance and perhaps in nature. We are not able to say whether one kind of fat can take two forms, although we are disposed to think that it does not, and that the globular and granular forms of fat may well be different forms, for example, the globular being perhaps neutral fat and the granular being soaps or yet other combinations.

In the fourteen cases which are definitely pathological, large globules of fat were always present, small globules frequently (eight), and granules rarely (three). If our supposition that the granules are quasi-pathological, be a correct one, it is likely that a liver so damaged as to be loaded with fat globules, would be perverted in function so that many of its cells would show fat granules; but the absence of these granules in eleven of fourteen cases seems to indicate that the two conditions do not necessarily arise from the same cause, or under similar circumstances; or else that the liver cell degeneration has advanced so far that it has passed the stage in which granules are to be seen.

The Globular Form.—There is no reason to suppose that small and large globules are different, for every gradation of size can be found, and globules appear to have the power of fusing just as they do in fluid; nor is there any reason to suppose that they exist outside of the cells, until such time as having distended the cells, they appear to have burst these and to have fused with other globules in inter-cellular spaces. In the light of the views held to-day as to the nature of the cell wall, namely, that it is a colloidal concentration of protoplasm rather than a real membrane, we find it hard to see why this so-called membrane should be resistant long after the cell protoplasm has been replaced: we incline to think that the signet-ring cell may gradually merge into a mere mass of globular fat, the nucleus disappearing. The most potent argument for this view is the huge size of fat globules in the liver. A digression may be made here to remark that in the case of globules of fat, large but not larger than the normal cell, the existence of this fat is consistent with the life and usefulness of liver cells. Whether the signet-ring cells functionate as liver cells we cannot say, but liver function can apparently be performed when no normal liver protoplasm other than nuclear can be made out in the section. It is well to make a mental correction of the apparent functional power of fatty liver tissue, because the tissue probably always seems more injured than it really is.

The fourteen cases of intense fatty change in this series do not arrange themselves naturally: twice they are alcoholic (a condition in which intense fattiness is well known to be almost, if not quite universal), seven times septic, five times toxic as the result of chemical poisons (compare the well-known effect of phosphorus), and once uremic. The last named was in no sense septic, and suggests that sepsis is not a necessary cause of this state: yet the fourteen cases uniformly exhibit a metabolic disturbance caused by a chemical or otherwise toxic agent. This observation does little more than support our previous supposition that fat is an evidence of the inability of the liver to perform satisfactorily one of its perhaps lesser functions, viz., the metabolism of fat. Carrying this statement further, we would expect to find that the mere process of disease-produced death, would, in itself, be attended by a lowering of metabolic power, so that all livers, save those found after instant death by accident, might be expected to show some fat; and so it appears to be.

Distribution of Large Globules.—Large globules occur most frequently in all parts of the lobules (twenty-seven): when one part only of the whole lobule is affected it is oftenest the peripheral (twelve), next oftenest the central (six), the middle zone never being the only part affected. If we count all combinations, globules are peripheral in forty-six, central in forty, middle in thirty-one. No generalization from this statement is possible, unless it may be said that the part which has the best double blood supply, viz., the mid-zone, is least often affected, and the change is not definitely connected with an over-supply of fat from the portal blood, otherwise the mid-zone would be more liable than the central. More likely does it seem that the mid-zone, which has, in addition to its portal supply, a supply of oxygenated blood from the hepatic artery, is by reason of its better supply of oxygen able to use up any excess of fat. Further, the central zone being next in order to receive oxygenated blood, is freer from this form of fat than the peripheral zone, which has only its portal supply.

Granular fat, on the other hand, we suppose indicates degeneration of the protoplasm, a condition that is not bettered by an increased oxygen-supply. Nor was the chronic passive congestion of the nutmeg liver a predisposing factor in those cases where the central zone was much affected; this observation at first sight appears to conflict with what we have just stated, because stagnated venous blood is the least oxygenated of all, but our supposition is not that lack of oxygen caused the defect, but that plentiful oxygen remedies it.

Irregular Areas of Large Globules.—In one case, groups of large globules appeared quite at random. It must be kept in mind that the knife may carry isolated globules across the section into different parts of the tissues, but this is generally readily recognizable. Twice, however, we have found a remarkable state of affairs: a definitely bounded macroscopically yellow area has proved to be made up of huge globules, the mass looking microscopically like a lipoma; however, in the liver tissue nearby globules of various size in the cells have indicated that the condition was one of gradual cellular acquirement, although we have no idea of the nature of the process which permits such a complete metamorphosis of localized pieces of tissue. These masses comprised many lobules, and their boundaries were those of lobules, a part of a lobule not entering into this state while the rest remained healthy.

Granular Fat.—Granular fat occurred more often than the globular, in the proportion of eighty-seven to fifty-eight. Granular fat alone occurred in forty, globular alone in eleven, while both were combined in forty-seven instances. The usual form is a slight distribution of granular fat throughout the lobules, while with this change are often united various distributions of globules. This universally distributed granular fat we suppose to be the true fatty degeneration of the liver (the word being used to designate a condition comparable to that found in the heart, and without prejudice to the question whether it is ultimately true infiltration or protoplasmic transformation). Whether these granules are a special form of fat, or whether they can coalesce and form globules we cannot say, but we think that they cannot coalesce. The occurrence of such universal granular fat appears to be consistent with otherwise perfectly healthy liver tissue, judged by the microscopic appearance, with ordinary stains.

When the granules are not diffuse but are limited to one or more zones of the lobule, they are most often central (ten), less often peripheral (two), and never median. If these granules have to do with ill nutrition or slight intoxication, as we suppose, it is to be expected that the part of the lobule doubly supplied with blood should be most free from them.

The appearance of fat when it occurs in the granular form is so distinct from the globular type that there is no difficulty in recognizing the condition. The character of the granules, whether in the peripheral or central zone, is the same. The granules first appear as very minute points distributed in the protoplasm of the liver cell without affecting the position of the nucleus. The granules later increase in size and also in number, until the cytoplasm is loaded with these discrete bodies, which have little if any tendency to coalesce. In no instance was it found that the quantity of fatty granules altered the central position of the nucleus, nor was the degeneration associated with any nuclear change.

Fat Globules and Granules in the Same Lobule.—In a fair number of cases it was noted that in the same liver lobule there appeared a deposit of fat, both of the granular and globular type. When these deposits were thus associated, the granular deposit was present in a diffuse form, while the fat globules were most commonly found in the peripheral zone. Such cases we describe as fat distributed diffusely in the lobule. The deposit of granular fat in the periphery was similar to the deposits in which globules alone were present in the lobule, and we could make out no direct association between the deposit of the globular and granular forms.

In other instances it was found that the granular deposit was predominant in the lobule, and only here and there was a cell or a small collection of cells containing fat globules. In these cases the globular deposit appeared to select no particular zone, but occurred sporadically in the lobule. These globular deposits were often very few, only one cell in a lobule, which was otherwise crowded with granular fat showing this deposit.

It is probable that, where there is a considerable deposit of fat in one or other zone of the liver along with the granular deposit in the other zones, we have to do with two separate pathological conditions, the one being superadded upon the other.

Granules and Globules of Fat Appearing in the Same Cell.—As we have noted above, fat may appear in the granular and globular type in the same lobule, and in some instances it appeared as if some of the granular deposit had coalesced to form globules. In only a few instances was it noted that definite small globules were present in a cell, while granules were also to be noted in the protoplasm of the same cell. This appearance was found the least frequently. It was sometimes noted that the globules in these cells had the same dark staining characters as were seen in the granular fat deposit. These cells containing both granules and globules had no fixed situation in the liver lobule, but were to be found in any of the three zones.

Fat in the Central Zone.—Our sections show that when we are considering the central zone alone, the fat found here is oftener granular (thirty-two), less often globular (sixteen). If our supposition that globular fat is a "physiological" phenomenon be a correct one, the difficulty is not to see why globular fat should be the rarer form in the central zone, but why it should be there at all, especially when the mid-zone is repeatedly free from it; yet we found this condition in six cases.

Is there a deposition of fat characteristic of uremia? In thirteen cases of uremia the fat was extreme once, considerable nine times, slight three times; it was diffuse four times, and the single areas affected were central (nine), peripheral (seven), middle (six); five times granular, four times granular and globular, and three times globular. From this observation, we are compelled to think that there is not a characteristic distribution of fat in cases of uremia.

It is of interest to note that of the sixteen cases in which the fat appeared in the central zone alone, thirteen were in females. Five of these were associated with recent pregnancies, three with uremia, one with pus tubes, and four with various infections.

CONCLUSIONS.

1. Nearly all livers at autopsy contain fat which is histologically demonstrable.

2. Fatty substances in the liver appear chiefly in two forms, as small granules and as globules of various sizes.

3. We suppose that granules indicate the (protoplasmic) change commonly spoken of as fatty degeneration, and that globules of small size are excess of fat, stored up by reason of some pathological change which may be merely temporary.

4. Fat is oftenest central, least often in the mid-zone.

5. A heavy deposit of fat is compatible with a competent liver.

6. Intense fattiness, generally globular, occurs with intoxications of bacterial and chemical nature, as well as in cases where a complex toxin is manufactured by the body-cells.

7. Granular fat occurs oftener than globular: it affects most often the central zone; globular affects most often the peripheral.

8. "Accidental" masses of globular fat are found at times, and appear to follow no rule of position: these are comparable to lipomata, which are evidently the result of a pathological process.

9. We found no deposit of fat characteristic of uremia.

10. We know of no analysis of the liver fats in the granular and globular states respectively: it seems to have been taken for granted that the deposits were one and the same. To determine the relationship, if any, between these two forms should be the basis of investigation.

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The Germicidal Action of Metals and its Relation to the Production of Peroxide of Hydrogen.

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(Communicated by J. G. Adami, F.R.S. Received June 12,—Read June 24, 1909.)

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That sundry metals possess not merely a distinct inhibitory action upon the growth of moulds, bacteria, and other micro-organisms, but even possess germicidal properties has been known for a long period, and there have been numerous observations demonstrating this action. The earliest observation known to us is that by Raulin in the seventies, upon the remarkable inhibition produced by traces of silver upon the growth of the *Aspergillus niger.* More recent work has in general followed the lines of the experiment employed by Miller, in his observations upon the bactericidal properties of gold and various alloys employed in dentistry; namely, plate cultures have been made of various micro-organisms, and into the inoculated culture media, while still fluid, small pieces or plates of the metals have been dropped, the presence or absence of a clear surrounding zone indicating arrest of growth of inoculated bacteria. As a result of these observations, it has been shown that certain metals possess inhibitory powers. More than this, according to Behring, reinoculation of the clear zone after the removal of the metal may lead to continued negative results. This would indicate that not merely is there inhibition, but an alteration of the gelatin, and suggests strongly the presence there of substances having a distinct germicidal effect. A full study of this nature was made by Bolton. Other observations (Ficker, Von der Dois, Clarke and Gage) have been along the lines of either employing metal vessels to hold the inoculated fluids, or placing strips of the metal in glass vessels holding water containing known cultures of bacteria. In these experiments also, metals like copper, zinc, and silver have been noted to possess distinct bactericidal effects. But in the above observations there has been no adequate attempt to determine the means whereby the metals exert their bactericidal action. Leedham-Green, however, made the observation that iron was inert or active according as to whether the conditions of the experiment prevented or permitted free oxidation of the metal. It was through oxidation that inhibitory action was brought about. The general opinion has been that there is a diffusion of the metal into the water or other medium, and that in some direct way the metal arrests the growth or actually brings about the death of the micro-organisms.

Certain observations made by Prof. Barnes, and a recent paper by Barnes and Shearer, have opened up the possibility of gaining a more direct knowledge of the mechanism by which these metals act, and the present series of observations owes its origin to Prof. Barnes. Barnes and Shearer working with aluminium showed experimentally that aluminium foil in water containing dissolved oxygen generated peroxide of hydrogen according to the following hypothetical equation :—

$$2Al + 6H_2O + 6O = Al_2(OH)_6 + 3H_2O_2.$$

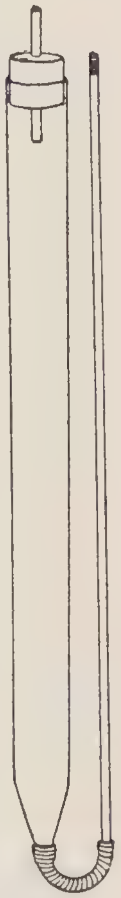
Concerning the production of the peroxide they write as follows:—"We placed pure aluminium sheet in ordinary pure distilled water open to the air and thoroughly charged, and after standing for a few hours we applied the well-known potassium iodide test for peroxide with marked success. Having obtained evidence of the production of the peroxide, we found that the yield could be increased by using considerable quantities of aluminium foil in small pieces, and leaving it for two or three days in water, through which air bubbled." They further showed that the production of peroxide of hydrogen depended upon the presence of dissolved oxygen, and subsequently that zinc acted in a manner similar to aluminium, and that with copper there was no production of peroxide of hydrogen.

The germicidal action of hydrogen peroxide is well known. It became, however, a matter of interest to observe in the first place in connection with those metals which in water gave origin to hydrogen peroxide, how far their bactericidal properties bore relationship to the presence of the peroxide, and, in connection with the metals causing no development of peroxide, whether any cause could be determined for their activity. The problems to be solved demanded the development of a series of experiments; in the first place, upon the maximal production of hydrogen peroxide through the agency of the pure metals, and later the effects of the metal in water containing dissolved oxygen, and lastly in oxygen-free water.

After some preliminary investigation during which many experiments were performed, the technique to be described was adopted, and, although some of the results here made use of were obtained prior to the improved method of investigation generally adopted, subsequent experiments showed them to be sufficiently accurate as compared with the newer method, the results varying slightly in degree, but not in kind. The metals used in the experiments were pure and were supplied by Prof. Barnes. The water used was the Montreal tap water, which is a soft alkaline water derived from the Ottawa River, containing variable, but in general small numbers of natural micro-organisms.

The medium used was plain nutrient agar, and the plates were incubated at 37° C. In the later experiments a medium containing iron, esculin and taurocholate of sodium, such as has recently been described by Prof. Harrison, was used. *B. coli* were added to the water drawn from the tap, so that the number of organisms very greatly exceeded that found usually in contaminated water.

A tube 40 cm. long by 3·5 cm. in diameter was drawn out to a nipple at one end. Over this was fitted a piece of rubber tubing with a section of glass tubing fitted on to the rubber at its distal end (see figure). The rubber was of such length that it was possible to attach the smaller tube to the side of the large one. The small tube extended above the level of the large one, and was, when sterile, plugged with cotton wool. The whole was then relatively sterilised by passing water at 70° C. through it for one hour. The metal having been previously cleaned and thoroughly washed in running water was dropped into the tube by the unsterile hand; 155 c.c. of the inoculated tap water was then added, and the apparatus attached to a Sprengel pump, and air bubbled through the water for a period of one hour. At the end of that time the rubber at the base of the large tube was pinched with the fingers, the attachment to the Sprengel pump disconnected, and a quantity of water removed from the larger tube by means of a sterile pipette, plated on agar, 1/20 c.c. in each case, and incubated at 37° C. for 24 hours, when the plates were examined and compared with plates to which a similar quantity of the untreated inoculated water had been previously added. In the tables these are referred to as Plate 2 and Plate 1 respectively. For determining the amount of peroxide of hydrogen present Bach's test was employed, as well as the potassium iodide test.



Control Experiment.—Using the above technique, inoculated water, containing *B. coli*, was bubbled for a period of one hour without the addition of any metal. There was slight, if any, reduction in the number of organisms in the water. The simple bubbling of air for one hour through water containing *B. coli*, then, does not cause any noticeable reduction in the number of organisms present in the water.

Effects of Metal without "Bubbling."—The following experiments were performed to see what effects metallic aluminium, zinc, and copper had upon bacteria when placed in contact with water at rest containing *B. coli* in the apparatus described. The results are shown below. It will be observed that zinc exerts some effect upon *B. coli* in one hour. It is noteworthy that aluminium and copper exerted none:—

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Hydrogen peroxide.
	c.c.	hour.			
Aluminium ... 50 grammes	150	1	Innumerable*	Innumerable	No reaction.
Zinc .... 50 ,,	150	1	,,	Slight reduction	,,
Copper .... 50 ,,	150	1	,,	Innumerable	,,

* At least 7000 to 8000 colonies per 0·05 c.c.

The water used in these experiments was obtained from the tap and held a fair amount of oxygen in solution. It is seen from this experiment that there is a bactericidal action (in the case of zinc) without demonstrable production of peroxide of hydrogen. In studying this it seemed possible that the oxygen in solution in the water played, nevertheless, some part in the destruction of the organisms, presumably by interaction with the metal.

Effects of Oxygen-free Water.—To investigate this the following experiments were performed. Tap water was again used, but previous to placing it in contact with the metals it was boiled for half an hour and then cooled:—

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Hydrogen peroxide.
	c.c.	hour.			
Aluminium ... 50 grammes	180	1	Innumerable	Innumerable	None.
Zinc .... 50 ,,	180	1	,,	,,	,,
Copper .... 50 ,,	180	1	,,	,,	,,

Here we see that there is practically no reduction with any of the metals.

Effects of Bubbling of Air on the Solubility of the Metal.—In the next experiment water was added to the three metals and air was drawn through the apparatus for a period of one hour, and subsequently the water was tested quantitatively for metal in solution.

Metal.	Water.	Metal in solution.
	c.c.	
Aluminium......... 50 grammes	150	Trace—hydrate thrown down.
Zinc.................. 50 ,,	150	,, ,, ,,
Copper .... 50 ,,	150	Less than 0·0015 part per 100,000.

It can hardly be possible that this small quantity of metal in solution can be responsible for any germicidal action exhibited in the experiments to follow. The work of Clarke and Gage would also point to the fact that it was not so. To investigate the point, however, the following experiment was performed:—

Aluminium, zinc, and copper were bubbled for a period of one hour in tap water, and the water was then withdrawn into a sterile bottle and allowed to stand overnight. These three portions of water were then inoculated with *B. coli;* plates were made immediately, and again in one hour. The results are given below:—

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.
	c.c.	hour.		
Aluminium... 50 grammes ...	150	1	Innumerable	Innumerable.
Zinc............ 50 " ...	150	1	"	"
Copper ...... 50 " ...	150	1	"	Slight reduction.

These results conform to the previous experiments, and were to be expected. Others have shown that small quantities of copper may at first inhibit the growth of *B. coli*, but that subsequently the results are very similar to those obtained when *B. coli* are added to sterile water in glass vessels and allowed to stand. The results with aluminium and zinc show that the amounts of these metals in solution exert no bactericidal action; that the small quantity of peroxide of hydrogen still present exerts no effective action.

Production of Peroxide of Hydrogen.—Quantitative tests of the amount of *available oxygen* in the water after air had been bubbled through for one hour gave the following figures:—

With zinc—

(1)...............	0·318 per cent. by volume, equivalent to 0·00096 per cent. hydrogen peroxide by weight.
(2)...............	0·299 per cent. by volume, equivalent to 0·00090 per cent. hydrogen peroxide by weight.

With aluminium—

(1)...............	0·252 per cent. by volume, equivalent to 0·00076 per cent. hydrogen pexoxide by weight.
(2)...............	0·195 per cent. by volume, equivalent to 0·00059 per cent. hydrogen peroxide by weight.

With copper......... None.

I do not need to emphasise the point that these amounts of peroxide per cent. can have little germicidal effect. Obviously the results to be noted below are not due to the mere presence of peroxide, but are due to some action with which is associated the production of the peroxide.

Germicidal Effect of Aluminium.—Aluminium strip cut into small squares and cleaned was then experimented with. The metal, in various proportions by weight, was bubbled with water containing *B. coli.* The results are shown below :—

Quantity of metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Peroxide.
	c.c.	hour.			
Aluminium, 125 grammes	150	1	Innumerable	1 colony	Present.
,, 100 ,,	150	1	,,	5 colonies	,,
,, 75 ,,	150	1	,,	1 in 48 hrs.	,,
,, 50 ,,	150	1	,,	1	,,
,, 25 ,,	150	1	,,	Many	Slight trace.
,, 25 ,,	150	1	,,	,,	,,

The first two experiments in the series were performed prior to a change in technique, and it was subsequently shown that 50 grammes of aluminium caused, depending upon the cleanliness of the metal, a good production of peroxide of hydrogen, and would generally give a plate free from colonies. A fair amount of peroxide of hydrogen is formed and a hydrate is thrown down. The hydrate has no opportunity of sedimenting, the bubbles of air keeping it constantly in motion and thoroughly mixed with the water. In taking the test water, therefore, the hydrate is thoroughly mixed with the water, thus excluding the possibility of live organisms being entangled in the precipitate and carried down with it, and thus not appearing on the plates. Quantitative tests for aluminium in solution showed only traces of the metal present.

Germicidal Effects of Zinc.—A series of experiments similar to those performed with aluminium were carried out with granulated zinc. The

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Peroxide.
	c.c.	hour.		hours.	
Zinc...... 125 grammes	150	1	Innumerable	24 none 48 a few	Strong.
,, ... 100 ,,	150	1	,,	24 none 48 one	,,
,, ... 75 ,,	150	1	,,	24 none 48 three	Fair.
,, ... 50 ,,	150	1	,,	24 none 48 two	,,
,, ... 25 ,,	150	1	,,	24 six 48 twenty-four	Slight.
,, ... 10 ,,	150	1	,,	24 thirty	,,

peroxide reaction was stronger than with aluminium, and the metal exhibited a more pronounced germicidal action.

With 50 grammes of zinc the plate was usually free from colonies, although occasionally a few colonies would develop. It was noticeable again that if the surface of the metal was not clean the yield of peroxide was slight and the reduction of the organisms in the water not so marked.

Germicidal Effects of Copper.—Pure sheet copper was then substituted for zinc. The following experiment sets forth the germicidal action of copper in this method of experimentation. It will be seen that copper exhibits quite a marked bactericidal effect, and that *without the production of peroxide of hydrogen.* Small quantities of copper could be detected in the water in about 0·0015 part per 100,000.

Metal.	Water.	Time.	Plate before, 1/20 c.c.	Plate after, 1/20 c.c.	Peroxide of hydrogen.
	c.c.	hour.			
Copper...... 125 grammes	150	1	Innumerable	None	None.
,, ... 125 ,,	150	1	,,	,,	,,
,, ... 125 ,,	150	1	,,	,,	,,
,, ... 100 ,,	150	1	,,	,,	,,
,, ... 75 ,,	150	1	,,	,,	,,
,, ... 50 ,,	150	1	,,	30	,,
,, ... 50 ,,	150	1	,,	33	,,
,, ... 25 ,,	150	1	,,	Numerous	,,
,, ... 10 ,,	150	1	,,	,,	,,

By reference to this table, it will be seen that 50 grammes of copper is not as strong a germicide as a similar quantity of zinc. Nevertheless, under the conditions of the experiment, it exhibits a strong germicidal action. This, as already noted, is: (1) in the absence of production of peroxide of hydrogen, and (2) under the conditions in which the amount of copper passing into solution is inadequate to explain the effects. We further have determined (3) that copper in tap water, with a free supply of oxygen, exhibits a stronger germicidal action than does ordinary tap or boiled water, and also (4) that when water is treated in the same manner, and for the same length of time, without copper being present, and is inoculated with *B. coli* in similar proportions, there is little, if any, reduction of the number of *B. coli* in the water.

Little attempt has been made in the past to associate the bactericidal influence of metals with oxidation or other effects produced by those metals, We learn that Novy and Hendry have carried out certain studies upon the production of "organic peroxides" by metals, and the effect of the same upon bacteria, but although some of their results were communicated verbally to a meeting of the Association of American Bacteriologists there has been no publication, nor am I acquainted with their findings. The only observation known to me is that by Leedham-Green already noted, upon the negative effects of unoxidised iron and the arrest of growth of bacteria when the iron is allowed to undergo oxidation. The general conception has been that the metal undergoes solution, and in this condition is directly active upon the bacteria.

As bearing upon the mode of action of metals, the results of the present investigations may be briefly summed up.

(i) Air drawn for one hour through water containing abundant colon bacilli exercises no perceptible inhibitory action upon their subsequent growth.

(ii) Relatively large amounts of pure zinc with large surface area placed in water, contaminated with abundant colon bacilli, and allowed to act for one hour, bring about a recognisable but not extreme destruction of the bacteria. Aluminium and copper, under similar circumstances, have no perceptible effect.

(iii) When the same experiment is repeated, with the one difference that the oxygen has been driven out of the water by previous boiling, none of these metals has any decided influence upon the subsequent growth of the bacteria.

It is thus obvious that such bactericidal activity of zinc as manifests itself is associated with the coincident presence of oxygen.

(iv) A much more intense bactericidal action is produced when air is permitted to bubble for one hour through water holding the colon bacilli in suspension, in the presence of aluminium, zinc and copper. Using a sufficiency of the pure metal it is possible to render the water completely sterile with all three metals, and that, when it contains abundant bacteria.

(v) In the case of zinc and aluminium, the sterilisation process is accompanied by the production of easily recognisable amounts of peroxide of hydrogen, and formation of hydrates of the two metals.

(vi) While this is the case, the peroxide itself cannot be regarded as the sterilising agent, and this because:—

(*a*) The amount of peroxide developed in one hour by bubbling air through the like quantity of sterile water in the presence of zinc or aluminium, while easily recognisable, is nevertheless very small in amount; a similar dilution of the peroxide added to a suspension of colon bacilli has no perceptible effect.

(*b*) Bactericidal action of the same or greater intensity is exerted by pure copper under like conditions of experiment, and *this with no associated recognisable production of hydrogen peroxide.*

(vii) It is thus evident (*a*) that oxygen must be present in order that these three metals, zinc, aluminium, and copper, may manifest bactericidal properties (in Montreal tap water), and (*b*) that in the process of interaction between the oxygen and the first two of these metals hydrogen peroxide becomes developed, whereas it is not detected in the case of the third.

Are we justified in drawing any conclusions as to the essential nature of the process of bacterial destruction that occurs in the presence of these—and possibly of other—metals? I am inclined to think that our general conception of the mode of production, and, it may be added, of the mode of disinfectant and sterilising action of hydrogen peroxide supplies the clue. We may lay down that the molecule of oxygen coming into contact with any of these three metals becomes dissociated with the liberation of free ions, of which, in the case of zinc and aluminium, some combine with the molecule of water to form the peroxide, and that it is these free ions, or in more old-fashioned language, "nascent oxygen," that is the essential agent in the bactericidal process. It is in this way that we explain the bactericidal activity of peroxide of hydrogen (for although I have laid down that in these particular experiments the amount of hydrogen peroxide developed was inadequate to explain the marked destruction of the bacteria, it must be remembered that this compound has notable disinfectant powers). We believe that the molecule of the peroxide coming into contact with the bacteria becomes dissociated, and that the liberated ion of oxygen is the destructive agent. It is quite possible that the hydrogen peroxide formed is thus a subsidiary agent in the bactericidal process where zinc and aluminium are present. It seems, however, simpler to presume that the dissociation of the oxygen molecule in the presence of water is the feature common to all three metals, and that in the case of the zinc and aluminium experiments the free ions of oxygen act upon the bacteria prior to, or, more accurately, in preference to, becoming associated with the molecules of water to form the peroxide, a certain excess, however, undergoing the latter combination. With regard to copper it must be postulated that the greater affinity of the free ions to the copper molecules than to those of water leads to non-formation of the peroxide or to rapid dissociation of such molecules as may become formed.

Why copper and zinc have diverse actions in the matter of the production of hydrogen peroxide I do not as a bacteriologist pretend to explain; I can but call attention to the facts.

To Prof. Adami my sincere thanks are due for suggestions and advice, as also to Dr. Oskar Klotz for his generous assistance, and to Dr. A. A. Bruere for aid given in the chemical analysis.

Note.—Subsequent to the above experiments, further analyses have been made, using boiled *distilled* water in the place of tap water. It was found that zinc in contact with distilled water, without the bubbling process, has a decided germicidal action. Copper has a variable effect, never as strong as that exhibited in the foregoing experiments. Aluminium usually corresponds in effect to that exhibited by plain boiled distilled water, which causes, in an hour, slight reduction in the number of organisms.

Away from the metal—that is, in water which has been in contact with the metal for one hour and then placed in a clean vessel—zinc water (distilled) still causes a reduction in the number of organisms. It will be seen that this is in line with the observations of Behring noted in our introductory paragraph. It is difficult to ascribe this to anything but the direct effect of the metal in solution, and yet, only the minutest traces of the metal are present, quantities which in tap water could hardly be responsible for the effects produced, and indeed would not produce them.

As bearing upon these somewhat divergent results it may be recalled, as a matter of familiar knowledge, that the action of distilled and of acid waters upon copper and other metals differs from that of the (usually) alkaline tap waters.

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CONCERNING COMPENSATORY HYPERPLASIA OF THE INTIMA.[1]

BY OSKAR KLOTZ.

Plate LXIX.

(From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pennsylvania.)

In 1885 Thoma set forth his views concerning the fundamental principles underlying arteriosclerosis. From the first Thoma's attention was concentrated on the intimal thickening which occurred in various arteries, and more particularly upon those developing in the aorta. His studies of these lesions were chiefly concerned with the general characters of the disease, to some extent with the nature of the tissues involved, and occasionally with the degenerative processes which develop in these intimal thickenings. According to his observations the intimal thickenings had common characteristics in their development, but only two types of the disease were recognized. In one instance the intimal thickening was diffuse and uniform, sometimes encircling the entire artery; in other cases nodular thickenings were evident, which might or might not be associated with the openings of smaller arteries. In his opinion the intimal thickening of both types developed in a process of connective tissue hyperplasia which was associated with a widening of the vessel lumen.

Thoma had previously convinced himself that shortly after birth there was an increase in the connective tissue of the intima of the aorta, extending from the mouth of the ductus arteriosus to and into the hypogastric arteries. His explanation for this development of connective tissue was that the loss of the placental circulation at birth led to the disproportion of the blood content to the vessel wall; that is, he believed that after birth the amount of blood in the vessels forming the foetal circulation was less than was

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present during intra-uterine life, and hence, to compensate for this diminished amount of blood, a new layer of connective tissue developed in the lumen, in order to make the tube narrower.

Although it has been found that there is a gradual development of intimal tissues with progressive age, no association can be demonstrated between the loss of placental circulation and the tissue hyperplasia. Nor can we support Thoma's findings that this new tissue in the intima is confined alone along the path of the fetal circulation. Moreover, the hyperplasia which occurs in the intima often involves elements other than connective tissue. Furthermore, the principle of compensation between the fluid of the body and the fixed tissues is usually balanced by other means. Under ordinary conditions the diminished amount of blood in the vascular system is readily compensated by the absorption of fluid from the surrounding tissues. How frequently during life must there be a temporary disproportion of the fluid blood to the arteries! Severe hemorrhages following trauma, hemorrhages occurring in typhoid and dysentery, and loss of blood occurring in cases of hemophilia all lead to this disproportion of blood content to the vascular system, and yet in these cases the disproportion is but temporary and the proportion can be easily reëstablished by the absorption of fluid. Moreover, in none of these diseases do we see a process of compensatory hyperplasia such as is described by Thoma.

This general principle of compensatory hyperplasia, which Thoma claimed for the connective tissue growth in the intima of the aorta of children, was also adapted by him for the explanation of the intimal thickening occurring in later life. Thoma discusses the subject in a general way only, and he avoids minute histological descriptions.

In 1886 Thoma carried out a series of experiments to demonstrate compensatory hyperplasia. He filled the fresh aorta in the cadaver, or aortæ recently taken from the cadaver, with melted paraffin under a pressure about equal to the blood pressure in the aorta. These injected preparations were kept at this internal pressure until the paraffin had thoroughly hardened. The aorta was then split open and the paraffin core removed. On examining these wax models Thoma claimed that the surface was uniformly smooth, and that opposite the sites of nodular sclerosis the paraffin showed no indentation such as might be expected from localized intimal thickenings.

Moreover, on examining the arterial wall, which had been subjected to the continuous pressure of the paraffin, he found that the intimal surface was quite smooth and free from the endarteritic irregularities such as are found in the collapsed arteries at postmortem.

Thoma further examined cross-sections of these arteries and found that not infrequently the media was thinned opposite the endarteritic plaque. He stated also, elsewhere, that the thickened intima might overlie an area of the media which appeared normal or even hypertrophied.

From his various observations Thoma deduced his well-known theory, the main features of which are briefly these: (1) the beginning of an arteriosclerosis, either endarteritis nodosa or diffusa, results from a weakening of the media which allows the artery to dilate either over a small circumscribed area or one involving a considerable portion of the arterial wall; (2) this dilatation of the arterial wall causes a widening of the lumen, which in turn induces a slowing of the blood current at this point. This slowing of the blood stream now acts as a local irritant leading to his so-called "compensatory hypertrophy of the intima."

Thoma's theories to-day are upheld by some and denounced by others. As we have improved our technique for pathological examination, it has been shown that some of his views are untenable.

During the foregoing winter, 1909, I repeated some of Thoma's experiments, but had little success in the use of paraffin as an injection fluid. I found that paraffin was liable to produce artefacts which were misleading. When paraffin was injected into the aorta, the globules of water adhering to the fresh vessel wall produced irregularities in the mold of the hardened wax. I also found that the paraffin, after it had set and when the pressure of the fluid paraffin without had no effect upon it, underwent a considerable shrinkage, producing other artefacts, and in my specimens it always showed extensive irregularities of the intima on the paraffin core. In no instance could I substantiate Thoma's claim that the lumen of an endarteritic artery was smooth and regular when under pressure.

However, remembering that there was considerable shrinkage in the paraffin while hardening, the possibility remained that when the vessel was properly distended at normal pressure or nearly so, the vessel wall was smooth, even with the presence of endarteritic plaques. It was, therefore, decided to use water for the injection fluid, and to fix the preparation by freezing. An apparatus was then devised so as to give any desired pressure, depending upon the height of the column of water above the aorta to be tested. When the proper pressure, equal to one hundred and sixty millimeters of mercury, had been obtained in the aorta, the whole apparatus was placed at a temperature of from 0° F. to —20° F. After an exposure of some hours the water in the specimen and apparatus was completely frozen and the character of the lumen was determined by sawing the frozen aorta into a series of small discs half an inch in thickness. The character of the lumen was easily distinguished in these discs, and impressions of the vessel wall in the distended state with the ice within were easily obtained. All the observations had, of course, to be made in the cold.

It was found that when the discs had been painted over with methylene blue and the excess stain had been removed with blotting paper, that accurate impression of the contour of the vessel wall could be obtained. Moreover, these impressions indicated with accuracy the contour of the lumen of the vessel while still under pressure. It is to be remembered that, as our specimens were filled with water under average high arterial pressure, the process of freezing, instead of relieving this pressure to some extent as is the case in the use of paraffin, actually increases the pressure, and hence would tend to obliterate still further the intimal projections and favor the views of Thoma.

In these experiments we were again unable to substantiate Thoma's contention. The intima with its white endarteritic thickenings could be quite readily distinguished from the media. Each of these endarteritic plaques formed a rounded bulging into the vessel lumen and nowhere could any weakening of the media be distinguished in the early intimal lesions. The small superficial

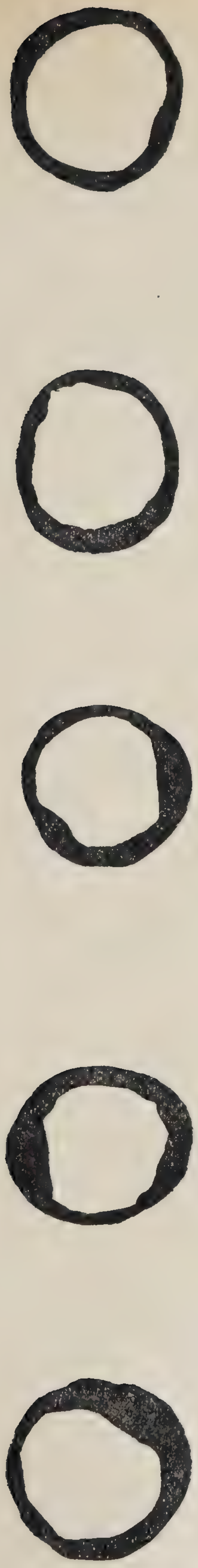

FIG. 1.

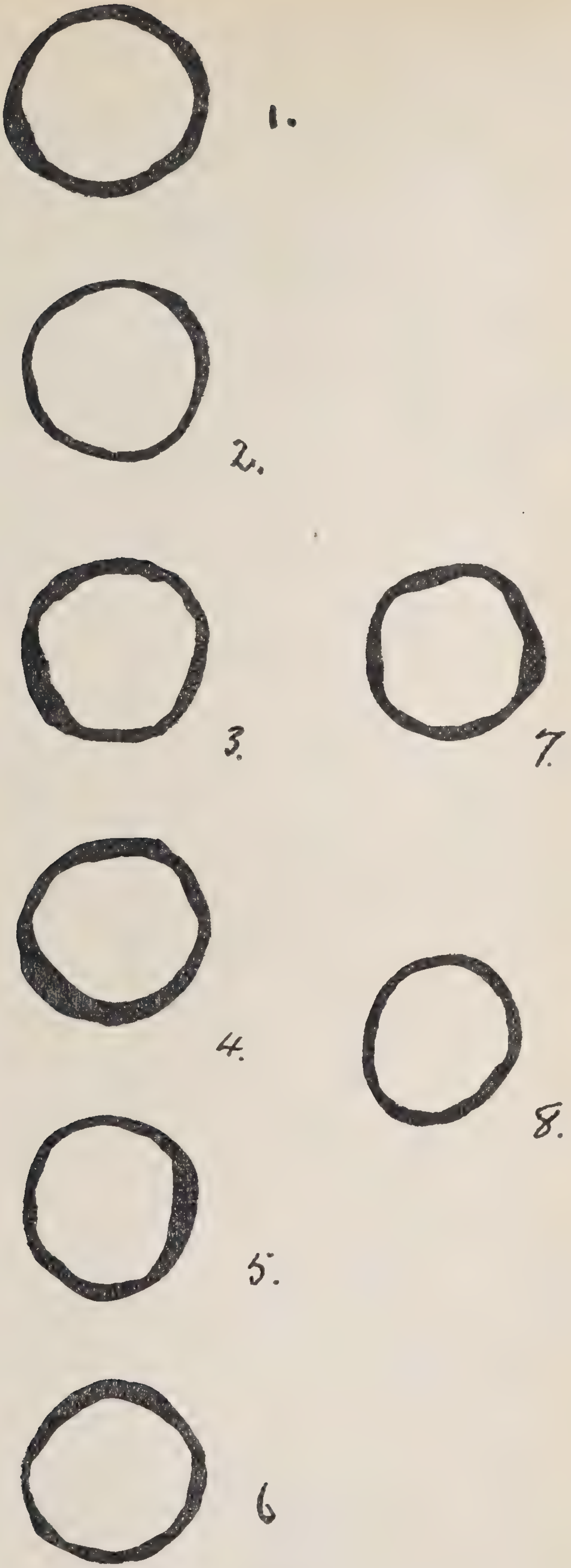

FIG. 2.

FIG. 1. Abdominal aorta of man aged 63, with extensive arterio-sclerosis and chronic endarteritis. Lumen much distorted.

FIG. 2. Aorta of man aged 75. Nos. 1, 2 and 3 are from thoracic aorta; Nos. 4 to 8 are from abdominal aorta. Chronic endarteritis with deep atheroma. Intimal lesions encroach into lumen. Media thinned in places.

fatty plaques which occur about the intercostal vessels were observed to rest upon the inner surface of the intima, like small masses of excess tissue. In many instances neither the deep intima nor the media showed any change in their structure or contour (Fig. 5).

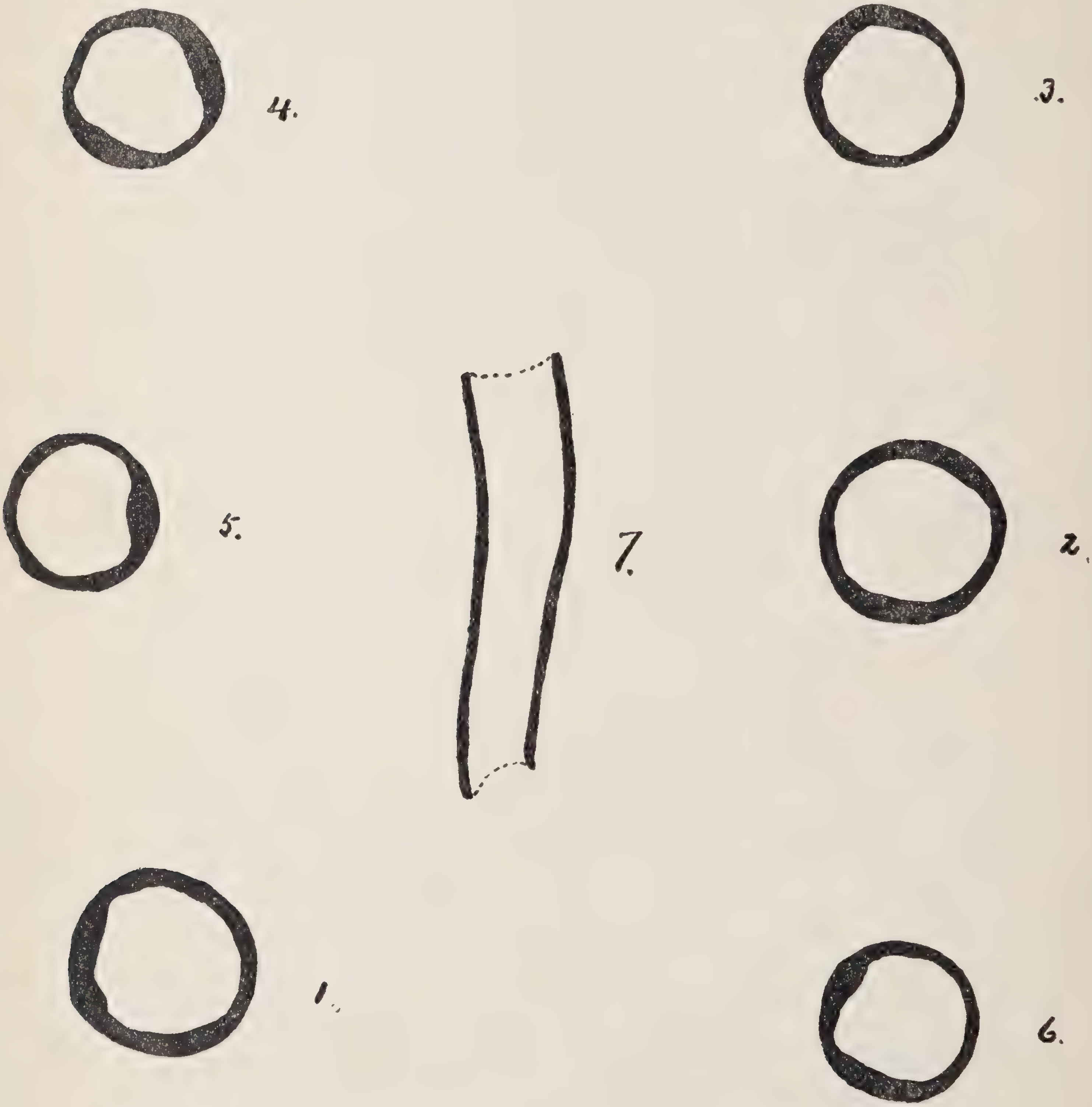

FIG. 3. Arteries from man aged 75. Nos. 1 to 6 abdominal aorta; No. 7, common iliac. Moderate diffuse and nodular endarteritis. Pouching in iliac obliterated by internal pressure.

Pearly thickenings of the intima without atheroma, the true endarteritis chronica nodosa, occupied the whole thickness of the intima and projected to a considerable extent into the lumen of the aorta.

The media was unaltered and formed a circular band of tissue with no evidence of bulging opposite the intimal thickening (Fig. 4).

On the other hand, in the cases of chronic endarteritis in which a degenerative softening had taken place in the deep structures, the media was found to be involved to a considerable degree and showed distinct evidence of thinning. In these specimens the deep intimal degeneration was so closely involved with a degeneration in the inner layers of the media that these two portions were fused, making it appear that the thickening and degenerative process was situated in the intima alone. The media was obviously thinned opposite the degenerative process, but there was nothing in the remaining media to indicate a primary weakening or change in these muscle fibers which could secondarily induce intimal hyperplasia to excess with degeneration (Figs. 1 and 2).

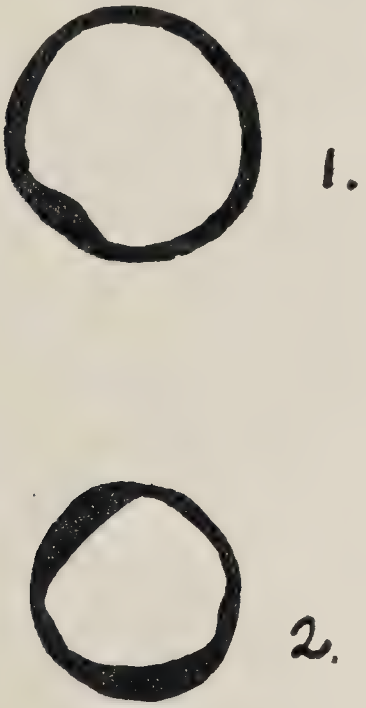

FIG. 4.

FIG. 5.

FIG. 4. Female aged 53. No. 1 thoracic aorta. No. 2 abdominal aorta. Showing early endarteritis in the thoracic portion projecting into the lumen, also irregular lumen of extensive sclerosis of abdominal portion.

FIG. 5. Female aged 26. Thoracic aorta. Showing early endarteritis. Endarteritic areas project into lumen of vessel.

By these same methods I also examined some vessels, particularly the iliac arteries, in which known lesions of the media were present. Some of these vessels showed typical Moenckeberg's arteriosclerosis with degeneration and calcification of the middle coat, while the intima showed little or no change over these areas. Macroscopically, and in the relaxed condition, these vessels showed small pouchings and transverse grooves on the inner surface. There were no nodular thickenings of the intima to be seen. When, however, these vessels were subjected to a hydrostatic pressure equal to one hundred and sixty millimeters of mercury and allowed to freeze, the longitudinal section of the artery showed quite a smooth intimal surface. The ridges and pouchings in the vessel wall had been obliterated (Fig. 3). Obviously here the more normal part of the vessel wall had, under pressure, dilated equal to the pouching present in the diseased areas. This is exactly what we should expect in comparing the results with the histological preparations. It has been repeatedly commented upon by various authors that the sclerosed and calcified areas of the media in Moenckeberg's disease diminish the elasticity of the media and fix the muscular tissues in a rigid state. In life this sclerosis takes place while the vessel is under pressure and hence the tissues become fixed and rigid while fully dilated. Under ordinary conditions of life, these sclerosed areas need not be more widely dilated than the adjoining more healthy arterial wall, as is shown in our experiments. Immediately on relaxing the blood pressure at death, the active and more healthy portions of the vessel wall contract, leaving the sclerosed portions as transverse areas to form the pouchings, as is seen at autopsy.

Here, then, we have a process exactly contrary to Thoma's theory. We find that in the areas where the media is diseased there is a pouching of the wall when the vessel is relaxed, and that these areas of medial disease are not covered by a thickened intima of compensatory hyperplasia.

But besides this macroscopic demonstration, we possess clearer evidence that the lesions of the intima are to a great extent independent of medial changes. Dividing the diseases occurring in the intima of arteries into the productive and the degenerative types, we shall, in this paper, confine ourselves to those lesions which lead to the development of new tissues. Nor is it within the province of this discussion to consider the final outcome of intimal hyperplasia, whether this leads to a process of degeneration or continues in the production of new tissue.

One meets not infrequently with specimens of endarteritis chronica nodosa in the aorta in which various grades of the disease can be recognized. The inner surface of the aorta is studded with pearly intimal thickenings of all sizes and in all stages, from the earliest beginning to the large plaques showing deep fatty change.

If, however, we confine our attention to those small islands of fibroses (be these of endothelial or connective tissue origin), which are just visible macroscopically, and study the microscopic characters of these specimens, we find that the diseased process is confined wholly to the intima with no change in the media. It is well to exclude, as far as possible, the presence of senile changes in the media of the aorta, and hence only vessels of adults under thirty-five years should be examined. By none of the known methods of staining can we demonstrate associated lesions in the media in these early processes of endarteritis. Fat and hyaline changes are wanting, calcareous salts have not been deposited, and the elastic fibers of the media show no inequality in staining. In short, we have to deal with a process which is confined to the intima.

This disease of the intima falls into two classes, according to the classification of Jores, (1) the hyperplastic, and (2) the regenerative.

Jores clearly points out that the hyperplastic thickening of the intima, which leads to an excessive development of the musculo-elastic layer, is a "hyper-physiological" process. There are many factors in the life-history of every individual which throw an increased strain upon this muscle layer of the intima of the arteries, and which, if not too persistent, lead to this natural hypertrophy. When, however, these factors are excessive, degenerative changes develop in the hypertrophied tissues. This result is a common, if not the usual finding in hyperplastic intimal thickenings in later life.

The regenerative lesions of the intima are processes taking place in the tissues superficial to the musculo-elastic layer, and occur quite apart from any changes in the deep intimal tissues. This regenerative process is regarded by Jores and others as a connective tissue proliferation of the nature of an inflammation but lacking, at the same time, the features of inflammation, as found in other tissues. A study of the earliest changes occurring in the development of these minute plaques shows that they are a result of the proliferation of the superficial cells. At the beginning, only a single type of cell, which by some is regarded as of endothelial origin, by others as of connective tissue origin, is found to take part in the proliferative change. Later, fine elastic fibrils are found to permeate the intimal plaque.

Such proliferative changes are most commonly met with in bacterial infections, and occasionally in association with poisons.

These lesions may progress to a considerable size before there is any evidence of change either proliferative or regenerative in the media, and no rule can be followed which will indicate the changes in the media during any particular stage of the disease. Moreover, it is not possible to determine any association between the development of these medial lesions and those of the intima. It would seem that, in these cases, the conditions in the media and in the intima arise wholly apart from each other, though they may result from a common cause.

From these cases we must clearly differentiate the lesions which arise in the media as a result of advanced intimal change. This is familiar to all and is quite a different process. When intimal lesions have been progressive and the thickening of this layer has become so advanced that nutrition is no longer able to pass from the lumen of the vessel to the innermost border of the media, nutritional degeneration, with a deposit of fat in the tissues, will take place. In these instances, the medial lesions are obviously secondary to an intimal disease.

Let us, for a moment, give our attention to the diseases affecting the media of the peripheral vessels and determine the part played by this coat in intimal hyperplasias. For this study the iliac, femoral, mesenteric or brachial arteries and their branches serve very well. Particularly in the case of the iliac and femoral arteries, can the medial diseases be studied from their first beginnings to the severest lesions.

The media of the peripheral vessels, as has repeatedly been indicated, is a functional muscular structure which has to do with the control of the blood supply of the part. Under conditions of stress the muscle tissue of the artery is active, and controls the amount of blood passing to the limbs or organs. Where the blood pressure and the velocity of the blood is increased, a smaller arterial lumen is required to give an equal amount of blood to the tissues, as under normal conditions. This diminution of the lumen is controlled alone by the media. Under ordinary circumstances the media can adequately control this blood supply, but when from various causes, such as hypertrophy of heart, Bright's disease, or excessive physical work, this blood pressure remains high for a long time, the media reacts by hypertrophy while the nutrition to its walls is adequate. When, however, the nutrition fails, this hypertrophied media slowly undergoes retrogressive changes. In the first degenerative changes of the media, the muscle fibers show an accumulation of fat granules in their protoplasm which continue to fill the cells until the muscle fibers undergo complete destruction. Later these areas of degeneration become calcified and in the superficial vessels are readily recognized as beaded tubes.

Such degenerations of the media are not accompanied by any inflammatory reaction and appear to be the result of over-strain or over-stimulation of the muscle elements. The condition can, in short, be considered as a process of fatigue of the muscle fibers coupled with nutritional disturbance. In consequence of the medial fatigue, there is a localized weakness of the wall, which is evidenced by the pouching that results in the areas of least resistance.

These progressive medial degenerations occurring in the peripheral vessels are readily studied in all the stages, and not infrequently several stages can be observed in the same artery. It is occasionally found that vessels show a considerable microscopic degeneration in the media before any pouching or dilatation results. This is probably due to the fact that the media had previously undergone an hypertrophy sufficient to withstand the heightened blood pressure even after some of the muscle lamellae had been destroyed.

These peripheral vessels showing medial degeneration show irregular conditions of the intima. In many cases the intima is found in a normal condition over lesions of various grades in the media. I have observed the successive changes in the media, from those showing the earliest sign of hypertrophy to the advanced changes in the media with pouching and calcification, without meeting with any condition of endarteritis nodosa or diffusa over the lesion. It is true that vessels with medial calcification not infrequently show a condition of endarteritis, but this endarteritis is found to bear no direct or secondary relation to the medial disease, save that both may be produced by the same injurious agent.

What is true of medial degeneration and dilatations of the peripheral vessels also holds true of the aorta. In the diffuse though not severe degenerations of the media in the aorta in old age, there is sufficient reason to believe that the arterial wall is materially weakened and that the vessel may be locally or diffusely stretched. Nevertheless, from this medial process alone we cannot predict what changes have occurred in the intima. Occasionally in such vessels the intima is without endarterial change.

Saccular aneurysms of the aorta not uncommonly show some thickening of the intima, but, as has been shown by others (Osler, Chiari), these aneurysms are most frequently the result of a syphilitic mesaortitis, and the intimal overgrowth is associated with the syphilitic process before an actual weakness or stretching of the vessel wall has shown itself. The mesaortitis is essentially an inflammatory process to which the neighboring intima reacts.

From our observations on the medial lesions occurring in the human blood vessels, we are unable to make any general statements associating these with the thickening of the intima, nor does there appear to be any "compensatory" reaction on the part of the intima due to medial weakening.

The experimental lesions in the arteries, too, have been of considerable value in the study of intimal hyperplasias. Some have contended that, as spontaneous disease may occur in the arteries of the experimental animals, or that the lesions experimentally produced were not comparable to the disease in man, no conclusions could be drawn from the work in experimental arteriosclerosis. Without discussing the right of this opposition, we need only point out that the study of the arterial lesions, as they occur in animals, has assisted to a great extent our knowledge concerning progressive and retrogressive changes of the various coats of arteries in general.

Various investigators studied the arterial lesions following mechanical injury (D'Anna, Malkoff, Israel). In all of these, varying grades of inflammatory reaction were obtained. The inflammation extended from the adventitia through the media to the intima. When healing was allowed to take place, there was a connective tissue replacement of the arterial wall, while the intima showed a nodular thickening over the injury. In some instances (Israel) the intimal thickening was followed by degenerative changes. It appears evident that in the intimal thickening we have to do with an inflammatory repair of the injured coat, although in the later stages all evidence of acute inflammation is wanting. It is probable that all vessels which are disturbed from their normal bed of *vasa vasorum* will later show inflammatory and degenerative processes.

Similar inflammatory changes of inconstant characters were obtained when large nerve trunks (sciatic) were interfered with (Bervoets and Fraenkel). However, it was later shown by Czylharz and Helbing that the vascular reactions were secondary to trophic lesions (ulcers) which occurred on the limbs of the animals. When no trophic changes were present, no arterial lesions developed. Hence the loss of nervous control of the arterial wall with the dilatation of the channels, was in itself not sufficient cause to produce intimal hyperplasia.

The arterial changes produced by the inoculation of adrenalin are interesting and have been thoroughly studied by some fifty-five investigators (Saltykow). The nature of the lesions is well known. In the majority of cases, the thoracic aorta was the site of predilection, though in a number of instances some of the peripheral vessels were also affected. These lesions most frequently consisted of primary degenerative processes in the media, affecting both the muscle and the elastic tissues. The muscular elements were first destroyed, and the whole vessel wall became thinner. In the early stages, with the loss of the muscle elements, the elastic fibers of the media were crowded closer together. Eventually the affected area became calcified and remained as an isolated mass in the middle zone of the media, with relatively healthy adventitia and intima on either side of it.

In only a few instances was any thickening of the intima observed over or associated with the medial degeneration. This thickening of the intima was in part a hypertrophy of the deep muscle coat of this layer along with some fibrous tissue overgrowth close beneath the endothelial surface. In some instances the hypertrophy of the musculo-elastic layer was quite remarkable, and in studying the various stages of the thickening of the intima in these lesions it was seen that the muscle hypertrophy preceded any development of connective tissue in the intima. Later, this development of muscle tissue in the intima was followed by degenerative changes simulating atheroma.

Although the medial degeneration was the most constant observation made by various investigators, none of them found sufficient evidence in their studies to associate relationship between the medial degeneration and the thickening of the intima. Ziegler, however, noted in his specimens, that the intimal overgrowth assisted, in some measure, the smoothing out of the depressions produced by the giving way of the media.

In other experiments we have more direct evidence of the production of intimal hyperplasias. Sumikawa found, when the femoral artery of rabbits was laid bare and was painted with a four per cent. solution of silver nitrate or with turpentine, that a localized inflammation followed in the tissues with an intimal thickening. The author concluded that the intimal reaction was a secondary one, following the general inflammatory process of the other coats.

Better results were obtained by the bacterial or toxin inoculations. In using various kinds of organisms, *B. typhi, Streptococcus pyogenes, Staphylococcus aureus* and others, it was found that considerable change occurred in the intima of the arteries, while the media showed little or no change (Saltykow, Crocq, Gilbert and Lion, Sumikawa, Klotz). Endothelial proliferations were seen early followed by an edematous or hyaline-like degeneration of the newly developed layer. In some cases a fatty degeneration was noted in the intima, so that the lesion resembled very closely the early atheroma of man. There was apparently a selective action on the part of the bacterial poisons which acted more particularly upon the tissues of the intima. On no account could the intimal hyperplasia, with splitting of the intimal elastic lamina, and its secondary degeneration be associated with a weakening or disease of the media. The hyperplasia of the intima was, in these infections, primary.

In my own experiments, which had been undertaken to eliminate as far as possible the action of poisons and toxins, it was found that increased work on the arterial wall, when carried on for an excessive period, had a double effect on the arterial tissues. On the one hand, it was found that, in those arteries which were subjected to the greatest stress and where that stress no longer acted as an irritant but as a damaging factor, the middle coat of the artery, and particularly the muscle cells therein, showed degenerative changes, even to destruction. The appearance of these vessels was quite similar to that which was obtained when adrenalin and other pressure-producing drugs were inoculated into animals. Quite similar to the adrenalin experiments, there was relatively little reaction in the intima over the medial lesions. On the other hand, in some of the smaller arteries, particularly in the carotid, where the damaging factors were not so severely felt, the artery reacted to some degree by a hypertrophy of its various coats. This hypertrophy was to be seen especially in the musculo-elastic layer of the intima. The size of the muscle bundle was distinctly increased and there appeared to be not only an increase in the size of the muscle cells but also in their number. This, in itself, assisted in thickening the vessel wall. Later changes were, however, prone to occur. In irregular areas this hypertrophied musculo-elastic layer became swollen and with ordinary staining appeared edematous. The tissue was loose and the connective tissue fibres about this layer had a hyaline appearance. In these areas great quantities of fat were readily demonstrated and it was found also that the internal elastic lamina had split into several layers, changes quite similar to those found in this band in arteriosclerosis in man. Other than the hypertrophy, the media showed no definite change, although in the areas where the fatty degeneration of the intima was extensive, a similar condition was found in the inner layer of the media bordering the intima. The mid and external zones of the media showed in no instance any feature which would indicate a weakening, and the slight amount of fatty change seen in the inner zone of the media was readily recognized as being secondary to the lesion in the intima (Plate LXIX, Figs. 1, 2 and 3).

Some have referred to the apparent intimal hyperplasia which occurred in transplanted vein segments as instances of Thoma's compensatory intimal hyperplasia. Since the valuable work of Carrel and Guthrie, indicating the manner in which arteries and veins might be successfully sutured, a number of operators have devised unique experiments on tissue transplantation. Fischer and von Schmieden have recently repeated the experiments of arteriovenous anastomosis and of the transplantation of a vein segment into the course of an artery. After allowing a complete healing of the sutured edges, the transplanted veins were examined after varying periods of time. The experiments were undertaken in answer to two questions: (1) Does a healthy vein become unduly dilated (varicosed) when under high pressure? and (2), Does the intima of the vein show a functional connective tissue hyperplasia? From their observation in their own experiments, they conclude that veins when under arterial pressure do not become unduly dilated, but after a time show a narrowing of the lumen, due to hypertrophy of the media by an increase in the muscle and connective tissue. The adventitia too was found to increase its connective tissue elements. The authors could find no compensatory hyperplasia of the intima, although nodular thickenings with inflammatory surroundings were occasionally observed. These nodules of the intima occurred most frequently about the sutures, and not in the most distended part of the vein, which was away from the suture line. In other words, the reaction in the vessel wall due to the increased function was found mainly in the muscle cells as an hypertrophy.

With reference to Thoma's views upon compensatory hypertrophy, it may be said that Adami has expressed views which, while subscribing to the idea that compensatory hypertrophy occurs, tend to ascribe it to other reasons. Both of the theories of Thoma, and those of Adami, assume a medial weakening antedating the growth of the tissue cells of the intima. Thoma contends that this weakening led to a dilatation of the artery with sufficient slowing of the blood current to cause reactionary proliferation of the intima; Adami, on the other hand, believed that the medial weakening and dilatation caused a tugging or stretching of the intima and its cells, and that these physical agencies were the stimuli for growth. However, the fundamental principle upon which both these theories are based, namely, a primary weakening of the media, remains unproved, and I would even say stands contrary to many observations on this point.

No one will doubt, I believe, that active work associated with an adequate nutrition will bring about hypertrophy or hyperplasia of many tissues. This increase of work may be, on the one hand, of a physical nature, or, on the other, of increased secreting function. In each case, the result is the same. While the nutrition remains sufficient for the needs of the tissue and the hypertrophied structures, a progressive increase in the number and size of the cells results. In other words, work in its various forms acts as a stimulus for cell growth, as long as the nutrition is adequate.

The tissues most affected in the arteries by the stress of physical agents, such as high blood pressure, are the muscle cells and, to a less extent, the elastic tissue fibers. When arteries are stimulated in this manner, these cells are the first to react, as is evidenced by the early hypertrophy of the media and of the musculo-elastic layer. These same cells are also those which suffer the effects of overstimulation, or fatigue, and in them degenerations appear early. In examining my specimens, Professor Adami, while recognizing the hypertrophy of the musculo-elastic layer, believed, nevertheless, that the overgrowth of the overlying connective tissue of the intima proper, as it showed no earmarks of inflammation, indicated a strain hypertrophy.

Much of this newly developed muscular tissue in the deep layer of the intima is followed later by very extensive fatty degeneration, so that much of this tissue is again lost. Over the hypertrophied muscle layer there is at times a development of a loose fibrous stroma, appearing hyaline or edematous. There is every evidence to believe that this superficial tissue, be it of endothelial or connective tissue origin, has developed secondary to the degenerative processes in the deep part of the intima.

Notwithstanding this, Adami believes that a share of the increased work of the arteries falls upon the superficial cells of the intima, and that the stretching of this lining causes a proliferation of the cells.

Concerning the reaction seen in the intima in syphilitic disease of the aorta, we incline to the view that it is secondary to the inflammatory processes of the media. Our increasing familiarity with syphilitic mesaortitis indicates that the lesions in the arteries are of an infective nature and that this infection lies in the adventitia and media. With this infective process there is truly a destructive process in the media, but there is also a concurrent inflammation which we feel convinced affects the intima.

The effect of moderately increased work upon the arterial walls leads to an hypertrophy of the muscle elements, giving way not infrequently to a later degeneration. The degeneration becomes most evident in the musculo-elastic layer which gives rise to atheroma. The growth of connective tissue elements over this degeneration is secondary.

When increased work exceeds the capabilities of the arterial tissues, degenerative changes set in from the first and no intimal hyperplasia is evident.

CONCLUSIONS.

We agree with Jores and others that not one but many factors may be at work leading to intimal hyperplasia. Among these factors may be mentioned infection, bacterial toxins, organic poisons, inflammation and increased arterial tension.

The theory of Thoma that the connective tissue developed in the intima is compensatory cannot be sustained.

From the evidence which we have at hand it is not possible to state that the proliferative changes in the intima are uniformly secondary to the weakening of the media.

Common influences may act simultaneously upon the media and the intima.

Progressive medial degeneration of the peripheral arteries (Moenckeberg's sclerosis) is the result of muscle fatigue coupled with nutritional disturbance.

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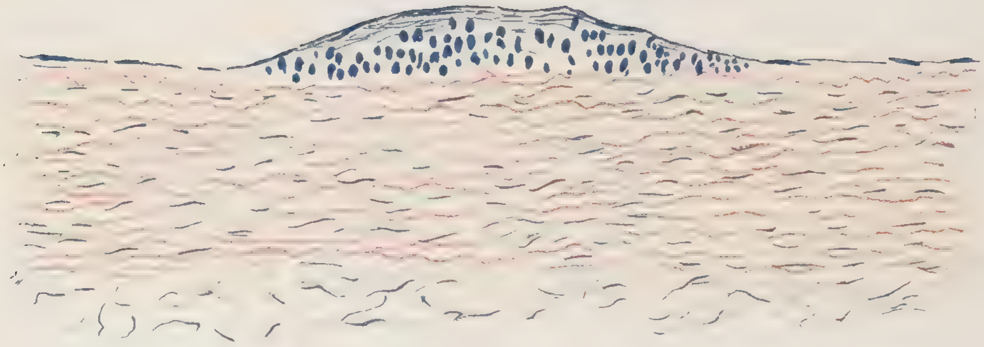

FIG. 1.

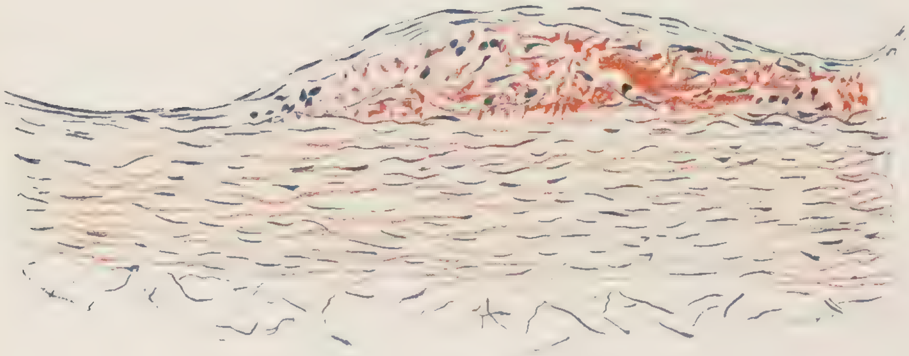

FIG. 2.

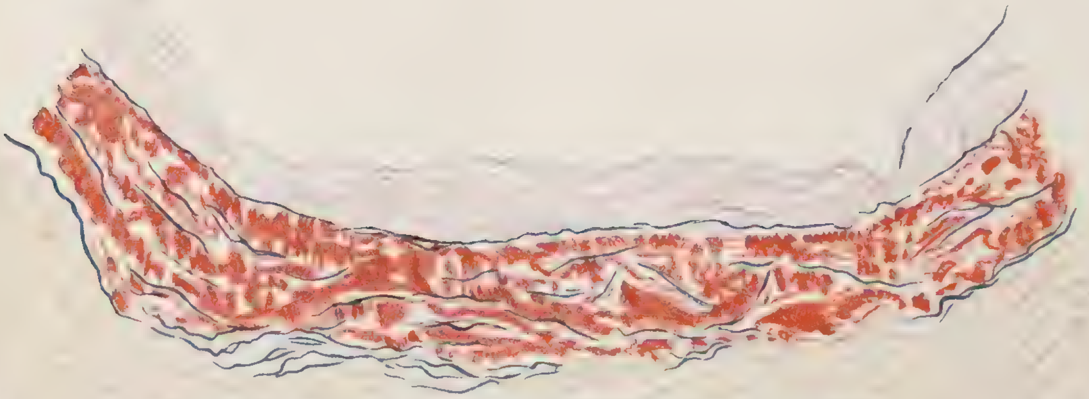

FIG. 3.

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EXPLANATION OF PLATE LXIX.

The tracings, including those in the text figures, are from impressions made from transverse sections of different aortas while frozen under pressure.

FIG. 1. Carotid artery of rabbit, daily inverted for five minutes for 120 days. Showing hypertrophy of musculo-elastic layer.

FIG. 2. Carotid artery of same animal as Fig. 6, in which hypertrophied musculo-elastic layer shows much fatty change.

FIG. 3. Carotid artery of rabbit treated for 135 days, in which the thickened musculo-elastic layer is very fatty, with a thickening of the subendothelial layer (endarteritis chronica).